Vol. XXIV November, 1947 No. 6

CEREAL CHEMISTRY

Published bimonthly by the American Association of Cereal Chemists
at Prince and Lemon Sts., Lancaster, Pa.

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Manuscripts for publication should be sent to the Editor in Chief. Advertising rates may be secured from and subscriptions placed with the Managing Editor, University Farm, St. Paul 1, Minnesota. Subscription rates, $6 per year. Foreign postage, 50 cents extra. Single copies, $1.25; foreign, $1.35.

Entered as second-class matter March 3, 1932, at the post office at Lancaster, Pa., under the act of August 24, 1912.

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Enriched FARINA	1.66	1.2	6.0	6.0
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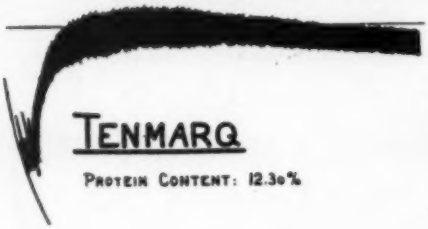

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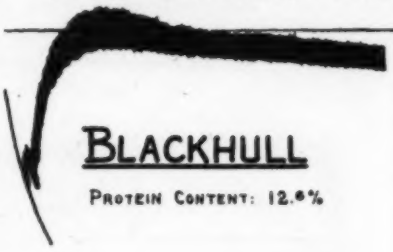

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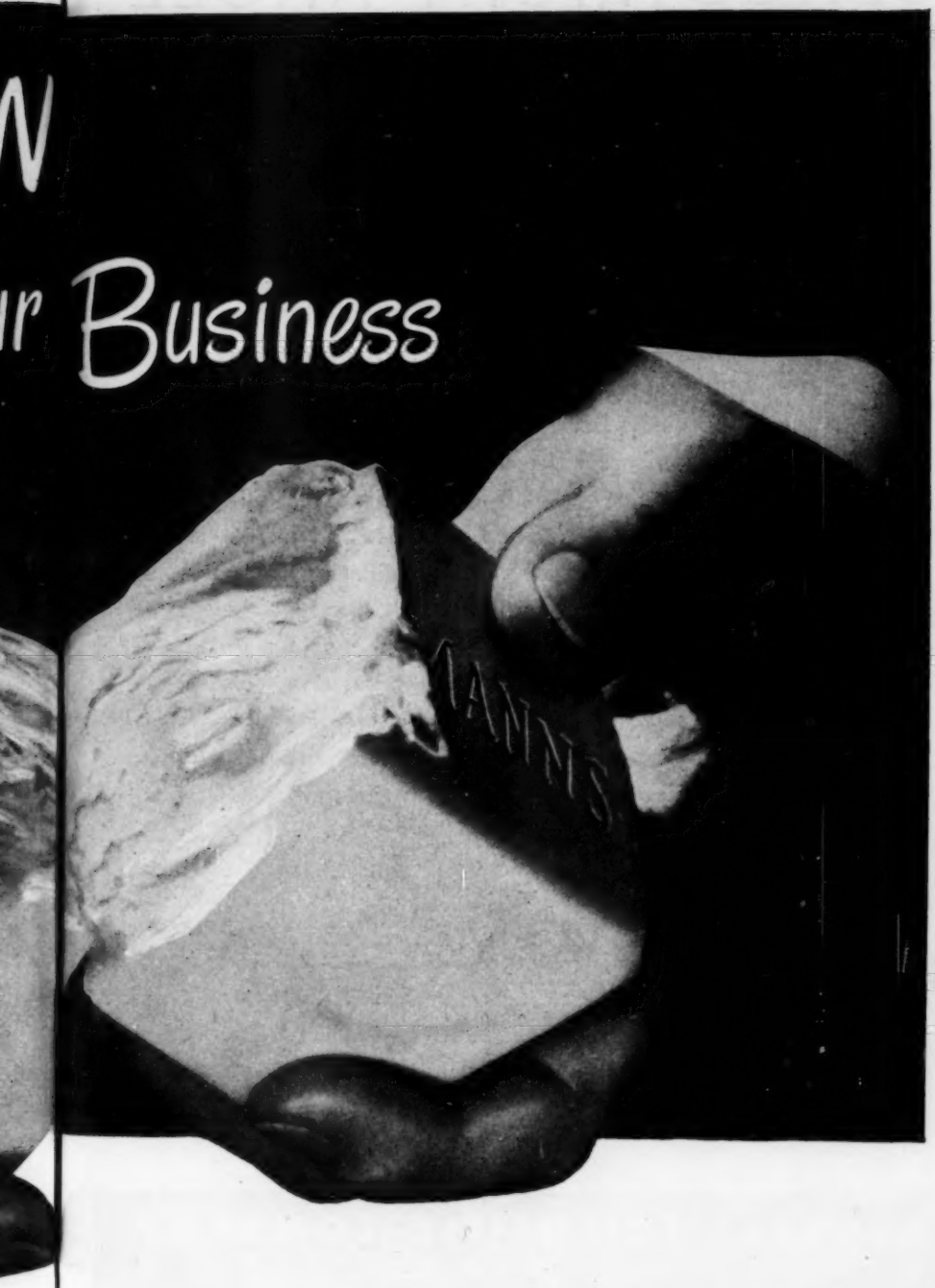

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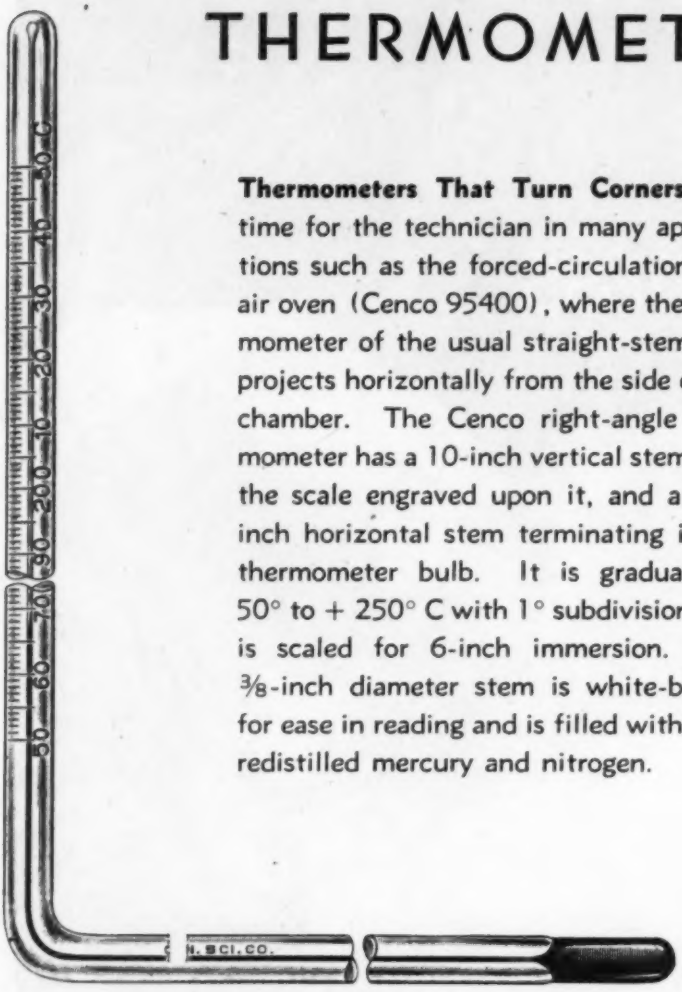

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CEREAL CHEMISTRY

VOL. XXIV NOVEMBER, 1947 No. 6

RELATIONSHIP OF THE PHYSICAL PROPERTIES OF WHEAT FLOUR TO GRANULATION[1]

FRANK W. WICHSER, J. A. SHELLENBERGER, and R. O. PENCE

Kansas Agricultural Experiment Station, Manhattan, Kansas[2]

(Presented at the Annual Meeting, May 1947; received for publication July 14, 1947)

In a previous investigation of flour granulation it was shown that the wheat endosperm particles passing through a flour cloth during sieving do not always approximate the size of the aperture openings of that cloth (Shollenberger, 1921). Recent work indicates that the particles vary in size from 150 μ to approximately 5 μ in diameter. LeClerc, Wessling, Bailey, and Gordon (1919) investigated the chemical composition of different-sized flour particles and showed that flour sifted through a fine silk bolting cloth had inferior baking properties to that sifted through coarser bolting cloth. These workers also found that flour sifted through a coarser flour cloth was only slightly better in baking properties than that sifted through the finest cloth, while the intermediate flour was found to give the best loaf. This suggests that the coarsest and the finest particles of flour had a detrimental influence on the whole flour. Maun (1927) and Kress (1929) substantiated the work of LeClerc *et al.* (1919). Pulkki (1938) and Swanson (1938) found that the flour particles passing through a fine flour cloth contained less protein than the coarser flour particles.

The difficulty confronting the past investigators in making a complete fractionation of flour into well-defined particle size groups was probably due to the mesh fineness limitations of silk flour cloths, and to the tendency for flour particles to agglomerate. The finest mesh silk flour cloth (25 standard) does not have aperture openings of a well-defined size or shape. The average aperture size openings of the 25 standard cloth is 63 μ, or approximately twice the size necessary to make a more complete particle size fractionation of flour.

Previous work on the relation of flour granulation to the chemical characteristics of flour has been limited largely to the reporting of

[1] This paper represents a portion of a thesis presented to the Graduate School of Kansas State College in partial fulfillment of the requirements for the degree of Master of Science.

[2] Contribution No. 142, Department of Milling Industry.

trends. It was the purpose of this investigation to make a complete fractionation of flour into several well-defined particle size groups, and to determine the physical and chemical characteristics and baking qualities of each group.

Materials and Methods

A commercially milled hard red winter, straight grade wheat flour was used throughout this investigation. The flour was fractionated

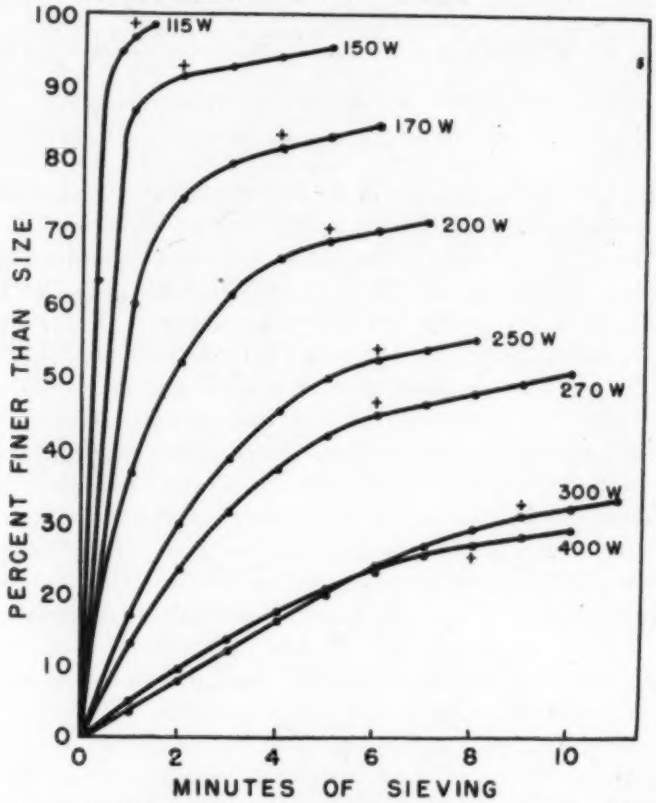

Fig. 1. Points establishing the optimum percentage of material through each wire testing sieve.

into 12 different particle size groups using a Ro-Tap sieve shaker equipped with W. S. Tyler standard wire sieves nos. 115, 150, 170, 200, 250, 270, 300, and 400. Following the sieving separation the fractions were subjected to an air elutriation treatment employing the Roller Particle Size Analyzer.

Separation by Sieves. The particle size distribution in the flour was accomplished by using one testing sieve at a time in the Ro-Tap shaker. The sieve was stacked upon a coarse wire screen carrying the under-sieve brush cleaners, and these two screens were then

stacked upon the pan. A small cloth cleaner was used for the top side of the testing sieve. A 50-g. sample was introduced onto the testing sieve and the sieving operation repeated at one-minute intervals. After each minute of operation, the top sieve was carefully removed and weighed. Extreme care was taken when removing and replacing the sieve not to disturb the "lay" of the material. This procedure was continued and the results followed by plotting a curve (Figure 1) of the percentage of throughs of the sieve against each min-

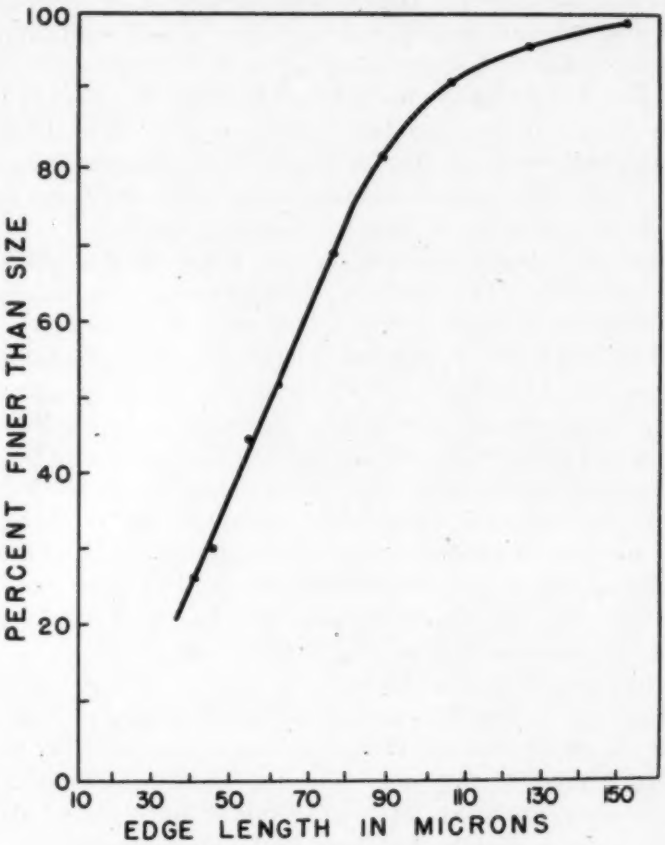

Fig. 2. Flour particle size distribution curve.

ute of sieving, until a point was reached in the curve after which essentially a straight line occurred. After this point was reached, additional material removed by continued sieving was probably the result of the reduction of particle size by attrition.

All remaining sieves were treated in the above described manner. The points, having been established when all of the sieving is completed, indicate the optimal percent of material through the sieve, and are illustrated by the granulation curve in Figure 2.

The sieving method just described was used to establish the flour granulation curve; but to obtain a larger quantity of the various particle sizes for thorough testing, a modified sieving procedure was employed.

A 50-g. sample of flour was sifted over the No. 400 mesh sieve for the number of minutes necessary to produce the optimal percentage of material through the sieve, as determined from the granulation curve. The overs of the sieve were then removed and another 50-g. sample of flour was sifted. The overs of the sieve were again removed and the procedure continually repeated until a large quantity of the sieve throughs was obtained. The next coarser mesh sieve, the No. 300, was used for sifting the material taken from the overs of the No. 400 mesh sieve, and this procedure was repeated for all of the succeeding coarser mesh sieves. Using the finest wire mesh sieve initially was necessary so that the coarser material would carry the finest particles to the mesh openings, permitting free bolting.

The particles passing through the No. 400 mesh sieve constituted the 0–38 μ fraction. The particles passing through the No. 300 mesh sieve made up the 38–46 μ fraction, and so on, until a complete fractionation of flour into its component particle size groups was accomplished.

Separation by Air. Flour particles have a tendency to agglomerate, and the agglomerates are not broken up entirely during the sieving process. Thus the accuracy of the particle size separation by sieving is limited. Also, sieving does not remove extremely fine or pulverized bran chips, dirt, or foreign material. However, the breaking of the flour agglomerates by air elutriation is quite effective. By this method it is possible to remove completely bran chips and all other contaminating material. The air separation principle was used on the particle size fractions obtained from sieving in this study.

The Roller Particle Size Analyzer (Figure 3) employs the air elutriation principle. The determination of removal of a particle size of powdered material below the 110-μ range is accomplished by means of a carefully regulated current of air. Any number of particle size fractions may be obtained. The air required for separation is regulated in accordance with Stokes' Law for falling bodies.

The analyzer, shown in Figure 3, consists of an air jet inlet (A), U-shaped glass vessel for holding the flour sample (B), oscillation connections for the latter, a series of four stainless steel settling chambers (9, 4½, 2¼, and 1⅛ inches in diameter) (C), a collector for the size fractions (D), and a gooseneck connector (E). The air jet inlet is threaded on the inside to receive an accurately bored nozzle.

The U-shaped sample tube oscillates approximately 200 times per minute under the action of leather-tipped fingers (F) mounted on a

motor-driven shaft. These oscillations are not free, but are constrained by the action of an abutment and spring. The action is such as to cause translatory-rotary contact between the flour sample and the air, which is highly efficient for the deflocculation action of the jet. The action prevents a haphazard shaking of the sample, which would be detrimental.

The cones of the settling chambers are tapped by a centrifugal tapper (G), so as to speed the downward movement of oversized material. These tappers consist of a pair of hammers rotating freely on

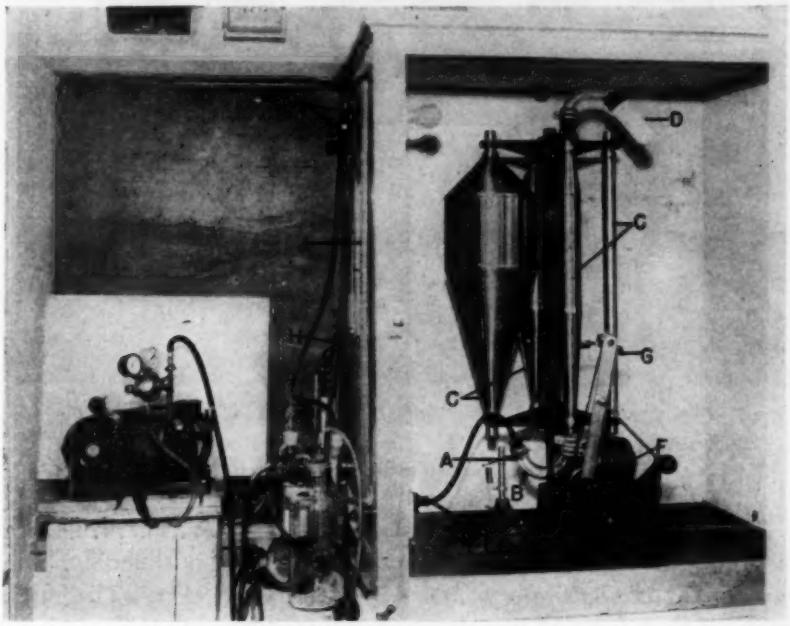

Fig. 3. Roller Particle Size Analyzer.

a shaft that is belted to the main motor shaft. The entire system is grounded to remove static electricity.

The apparatus has a carefully calibrated flowmeter (H). The entire range of air flow for flour is covered by two capillaries (I), which are interchangeable by means of a large bore three-way stopcock. A mercury manometer (J) measures the pressure drop across the inlet nozzle and provides a means for obtaining a flowmeter correction. This correction is applied in order to retain a constant pressure within the U-shaped sample tube for all air velocities.

Since the instrument was used as a "clean-up" measure on the particle size fractions obtained by sieving, the air velocity used was

such that it would remove particles up to the lower size limit of each fraction. Small particles removed comprise broken agglomerates, starch granules adhering to the larger flour particles, and all bran chips. The resulting flour fractions were of a well-defined particle size and were completely freed from any material other than pure endosperm particles of a stated size range. A comparison of the fraction particle sizes is shown in Figure 4. Microscopic observations were made as a size control on all of the fractions.

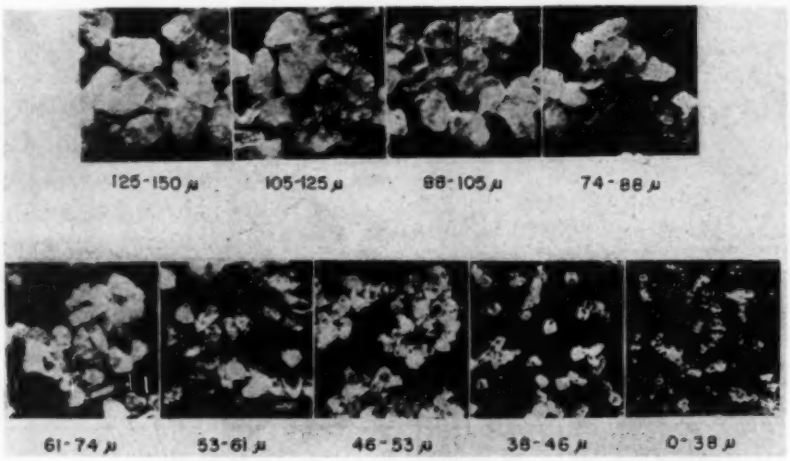

Fig. 4. Photomicrographs of the flour particle size fractions. Magnification: 35×.

Chemical and Physical Analysis. Analysis for ash, protein, and gas production was carried out by the methods outlined for wheat flour in *Cereal Laboratory Methods* (4th ed., 1941). The specific gravity of each fraction was determined by employing the pycnometer and method described by Sharp (1927), with air buoyancy corrections applied in each case. The amylograph was used as described by Anker and Geddes (1944), while the farinogram curve patterns were obtained in the usual manner. The valorimeter was used as described by Johnson, Shellenberger, and Swanson (1946) to determine the curve characteristics. The National-Swanson-Working recording dough mixer was used for obtaining the mixogram curve patterns. Johnson, Swanson, and Bayfield (1943) have fully discussed the theoretical and practical application of the mixogram to cereal research.

Each flour fraction was baked using the baking procedure employed by Johnson, Swanson, and Bayfield (1943), except that the absorption was determined by use of the farinograph. The rich formula was used.

Results and Discussion

Table I gives a summary of data for the relation of particle size to the various tests.

TABLE I

RELATION OF FLOUR PARTICLE SIZE TO ASH, PROTEIN, AND AMYLOGRAPH, MIXOGRAPH, AND FARINOGRAPH CURVE CHARACTERISTICS[1]

Particle size range	Ash	Protein	Amylograph maximum viscosity	Mixograph		Farinograph	
				Area under curve	Mixing time	Absorption	Valorimeter reading
μ	%	%	*Brabender units*	*cm.²*	*min.*	%	*units*
Flour	0.44	11.1	695	77.4	3	61.6	78
125–150	0.44	9.7	—	—	—	—	—
105–125	0.32	10.0	1020	71.7	3½	58.9	74
88–105	0.35	10.4	935	72.3	3½	60.3	77
74–88	0.36	10.6	880	72.3	3½	60.1	77
61–74	0.37	11.4	875	74.2	3½	60.6	76
53–61	0.39	12.3	830	79.4	3¼	61.7	66
46–53	0.43	13.5	735	83.8	3½	64.3	74
38–46	0.52	13.7	—	—	—	—	—
0–38	0.51	9.1	570	65.0	4¼	61.5	57
0–105[2]	0.44	11.1	695	76.2	3½	62.1	82
38–150[3]	0.41	11.9	695	74.6	3½	62.1	80
38–105[4]	0.41	12.0	845	79.2	3¼	62.1	85

[1] All values expressed on a 14% moisture basis.
[2] Flour with only the largest particles (105–150 μ) removed.
[3] Flour with only the smallest particles (0–38 μ) removed.
[4] Flour with the largest and the smallest particles removed.

Ash. A decrease in the size of the endosperm particle is accompanied by an increase of ash content of the particles. Since the particle size fractions have been subjected to air elutriation, where the removal of bran is completed, the resulting ash of the flour fractions is apparently an inherent characteristic of the particle in the fraction. The largest flour particles should then have the lowest ash content. This one fraction, however, contained such large chips of bran that an air velocity great enough to remove the bran would also have removed the flour particles. The 0-38 μ fraction is below the size of most existing flour particles. Thus it is composed largely of free starch granules and constitutes approximately 27% of the whole flour. Since the ash content of this fraction is high, it has a strong influence on the final ash content of the original flour.

Spectrographic Analysis. A spectrographic quantitative analysis for the elemental distribution as related to particle size is shown by the data in Table II.

TABLE II

RELATION OF FLOUR PARTICLE SIZE TO INORGANIC ELEMENTAL DISTRIBUTION[1]

Particle size range	Ash	Milligram percent in flour							
		P	K	Na	Ca	Mg	Mn	Fe	Cu
μ	%								
Flour	0.44	79.0	70.2	6.6	16.2	14.9	3.0	1.0	0.2
125–150	0.44	70.1	68.8	11.0	16.2	13.2	2.4	1.9	0.8
105–125	0.32	68.5	75.9	6.1	11.8	10.3	1.9	0.7	0.5
88–105	0.35	58.6	36.2	8.1	10.8	6.7	1.2	0.4	0.6
74–88	0.36	57.4	39.9	6.8	11.7	13.1	2.1	0.4	0.8
61–74	0.37	87.8	39.9	6.6	13.9	12.8	2.5	0.7	1.2
53–61	0.39	84.7	35.8	3.5	15.6	12.7	2.5	1.0	1.2
46–53	0.43	76.2	40.3	6.1	14.9	12.1	1.9	1.0	2.1
38–46	0.52	91.5	67.6	5.7	16.3	17.2	3.3	1.1	2.5
0–38	0.51	78.2	61.8	6.1	14.6	12.3	2.2	0.5	2.4

[1] All values expressed on a 14% moisture basis.

Potassium and phosphorus are the major constituents in the ash. Phosphorus is high in the particle size ranges of 0–74 and 105–150 μ and low for 74–105 μ. Potassium is high in the 0–38 and 105–150 μ particle sizes and low in the intermediate size material. These two elements do not appear to follow a trend of distribution according to the quantity of ash or particle size, although a lower quantity is seen in the intermediate size material. Small differences are seen for the other inorganic elements in distribution in the various fractions, although no trend is noted.

Protein. The relation of particle size to protein content is the same as with ash. A decrease in the size of the endosperm particle is accompanied by an increase of the protein content. The 0–38 μ fraction contains some free starch granules, which accounts for its low protein content.

Morris, Alexander, and Pascoe (1946) removed various zones of endosperm from the wheat kernel by using a dentist drill and found that the innermost zone produced a flour whose ash and protein contents were lower than any other zone. As the zones radiated out toward the bran coat, the flours increased in their ash and protein contents. Thus, the largest flour particles originated in the innermost zone of the endosperm, while succeeding smaller particles came from zones as they radiated out toward the peripheral layer. Apparently the innermost zone was more vitreous, remaining in larger particles. These larger particles were lower in protein content than smaller flour particles. However, Berg (1947) found that the vitreousness of the endosperm and the subsequent flour particle size does not depend upon the protein content.

Gas Production. The relation of gas production to the fractions having malted wheat flour added and to untreated fractions is shown in Table III. The production of gas was least on the untreated frac-

TABLE III

RELATION OF FLOUR PARTICLE SIZE TO GAS PRODUCTION

Particle size range	Gas production in mm. of mercury							
	Untreated				1% malted wheat flour added			
	4	6	8	24	4	6	8	24
μ	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*
Flour	306	333	366	535	499	595	666	1050
125–150	—	—	—	—	—	—	—	—
105–125	209	222	238	339	381	442	500	928
88–105	221	238	252	352	408	473	523	846
74–88	232	251	267	379	415	481	533	896
61–74	224	241	255	362	408	473	523	874
53–61	227	242	257	351	418	476	536	889
46–53	250	266	284	386	440	501	557	891
38–46	290	309	330	447	470	545	602	926
0–38	417	462	506	682	495	697	821	1141
0–105[1]	320	351	385	561	503	603	672	1055
38–150[2]	242	267	277	383	422	480	532	840
38–105[3]	243	265	279	387	433	505	555	861

[1] Flour with only the largest particles (105–150 μ) removed.
[2] Flour with only the smallest particles (0–38 μ) removed.
[3] Flour with the largest and the smallest particles removed.

tion whose particles were largest in size. With a decrease in the size of the flour particles an accompanying increase in gas production was obtained. This same relation existed when the fractions were supplemented with malted wheat flour. Since the increase in height of these curves is nearly all proportionate over the curves given by the unmalted fractions, there is no indication of a concentration of amylase in any one fraction. The difference in gas production is apparently due to the susceptibility of the flour particles to enzyme attack. With a decrease in size of the flour particle, the susceptibility of the particle becomes greater. The 0–38 μ fraction shows the highest rate of gas production in both untreated and malted fractions. This is probably due to a concentration of starch granules, some of which are ruptured and therefore are highly susceptible to amylase action.

Amylograph Curves. The relation of particle size to the maximum viscosity determined by the amylograph is shown by data in Table I. The viscosity is least for the smallest particle size fractions. With an increase in size of the flour particles there is an accompanying increase of the viscosity.

There was no evidence from either gas production data or amylograph curves of the differential influence of amylase activity in any one fraction as compared with another. Thus the marked differences in the viscosity curve heights are due to the various flour particle sizes. The 0–38 μ fraction is composed of many starch granules, some of which are ruptured from mechanical operation. Because of the increased surface area thus created, the amylase brings about the degradation of the starch granules more rapidly. The liquefying action of amylase is in opposition to the viscosity increase caused by gelatinization.

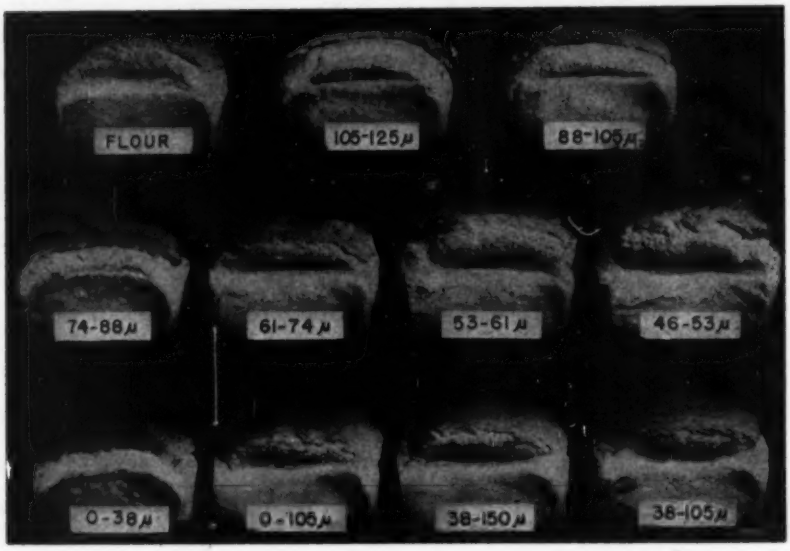

Fig. 5. Comparison of the bread baked from the various particle size fractions.

Farinogram and Mixogram Curves. The relation of particle size to farinogram and mixogram data is given in Table I. Only small differences between curves are noted except for the 0–38 μ fraction. Since the protein content of a flour is one of the most important factors in determining the farinogram and mixogram patterns, the low protein content of the 0–38 μ fraction accounts for the poor curve produced. The mixograms gradually increase in height as the particle size decreases, as shown by the area under the curves. This curve rise is attributed to a protein increase. There is also a general trend for the water absorption to increase with a decrease in the size of the particle. This can be attributed to an increased protein content and to the greater amount of surface area exposed as particle size decreases. The valorimeter value for each curve showed little relation to particle

size; however, on the flours from which the coarse material, fine material, and both coarse and fine material had been removed, the value of the curve is increased greatly.

Specific Gravity. The basis upon which the air elutriation principle is founded is that the same specific gravity exists for all particle sizes of flour. Thus a change of air velocity is required to remove the different-sized particles having a uniform specific gravity. Careful determination of the specific gravities of all particle sizes showed very little difference among them.

Baking Results. A comparison of the loaves of bread baked from the particle size fractions is shown in Figure 5 and a summary of the baking data is given in Table IV.

TABLE IV

RELATION OF FLOUR PARTICLE SIZE TO BAKING DATA

Particle size range	Loaf volume	Crumb color[1]	Texture grain[2]	External appearance (break and shred)
μ	*ml.*	*score*	*score*	
Flour	760	75cy	83-o	Good
125–150	—	—	—	—
105–125	775	84cy	78-o	Poor, half shell top
88–105	775	85cy	81-c	Poor, half shell top
74–88	775	88cw	82-c	Poor, half shell top
61–74	795	90cw	85-o	Fair, partially shell top
53–61	860	90cw	88-o	Good
46–53	920	88cw	90-o	Good
38–46	—	—	—	—
0–38	610	78cy	75-c	Very poor, half shell top
0–105[3]	785	78cy	86-o	Fair to good
38–150[4]	805	80cy	87-o	Fair to good
38–105[5]	835	80cy	88-o	Fair to good

[1] Crumb color—cy = creamy yellow; cw = creamy white.
[2] Texture-grain—c = close; o = open.
[3] Flour with only the largest particles (105–150 μ) removed.
[4] Flour with only the smallest particles (0–38 μ) removed.
[5] Flour with the largest and the smallest particles removed.

Perhaps the most important characteristic sought in a loaf of bread is loaf volume, assuming that the loaf grain and texture are good. Since loaf volume is directly related to the protein content of the flour, the loaf volume is low for the coarse flour fractions whose protein content is low. An increase in loaf volume is obtained as the protein content increases, or as the flour particle size decreases.

The largest particles had the lowest protein content, and produced shell-topped bread of poor volume and appearance. The smallest flour particles produced bread of largest volume with good grain and texture and excellent color. The larger flour particles produced bread with a creamy colored crumb, which indicates a higher concentration of the carotinoid pigments.

The flours with the coarse, fine, and coarse and fine particles removed produced loaves superior to those obtained from the original flour, and compared favorably with the 46–53 μ fraction whose high protein content produced the best loaf.

Practical Implication of the Study. Wheat tempering or conditioning must influence to a large extent the granularity of a flour because of the existing relation of particle size and zonal origin.

Air separation of stock in the milling process can assume a greater role for the selection of flour endosperm of improved quality. Air separation can eliminate the starchy, low protein, high ash material which tends to lower the baking quality of a flour.

Summary

A commercial hard wheat flour was fractionated by means of wire sieves and by air elutriation into 12 different ranges of flour particle sizes. Chemical and physical tests performed upon the various fractions showed a wide range in characteristics, which were related to the size of the flour particles. An increase of ash, protein, water absorption, gassing power, area under mixogram curves, and loaf volume was found, with a decrease in the size of the flour particles to the lowest limit of approximately 38 μ. The 0–38 μ fraction size was composed of some free starch granules with a slight overlap of the very finest flour particles, resulting in a fraction of low protein, low viscosity, reduced area under mixogram curve, and less loaf volume. This same fraction was high in ash and gassing power. No significant trend for inorganic elemental distribution as related to the quantity of ash or particle size was noted.

Protein content was the dominant factor influencing the quality of each fraction of the flour, and the ash content was shown to be an inferior yardstick of flour quality measurement.

Gas production measurements indicated that amylase activity was practically uniform throughout the various flour fractions and that gas production increased with decrease in particle size.

The carotinoid pigments are apparently concentrated in the coarser flour particles, since the finer particles produced a bread loaf whose color was creamy white.

Removal of the finest and the coarsest particles from flour enhanced greatly the remaining portion of the sample. The improved quality is not due to the more uniform granulation of the flour, but to the increased protein content of the sample, which results from the removal of the fine and coarse particles.

The specific gravities remained the same for all particle size fractions.

Baking results for each fraction were closely related to flour quality as determined by the various physical and chemical tests.

The relationship of particle size to ash and protein is indicative of the zonal source of these particles.

Acknowledgments

The authors are indebted to the Rodney Milling Company, Kansas City, Missouri, for the research grant which made this work possible. Dr. W. G. Schrenk, Department of Chemistry, Kansas State College, Manhattan, Kansas, carried out the spectrographic analyses.

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SPECIFIC SURFACE OF WHEAT FLOURS. I. DETERMINATION BY AIR PERMEABILITY METHOD

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(Presented at the Annual Meeting, May 1947; received for publication July 14, 1947)

A knowledge of the amount of surface associated with a given weight of a finely divided material has been of importance in studying various processes in a large number of industries. It is possible that this same type of knowledge may be of value to the cereal chemist in checking various milling operations and in correlating specific surface with other properties of a flour.

During the last decade two methods of determining the specific surface of finely divided materials have been developed. Low temperature adsorption isotherms, using nitrogen gas primarily as the adsorbate, have been used in determining surface areas of porous and finely divided materials by Emmett and Brunauer (1937), Brunauer, Emmett, and Teller (1938), and Harkins and Jura (1944). The other method is based upon the permeability of porous media to fluid flow. Lea and Nurse (1939), Gooden and Smith (1940), Blaine (1941), and Pechukas and Gage (1946) have all successfully used air permeability methods for measuring surface areas of powders.

The purpose of this work was to develop a method for the determination of the specific surface of cereal flours that would have a high experimental accuracy and would be adaptable for routine analysis. From consideration of the experimental technique of measurement, the air permeability method was chosen as the one most likely to achieve this purpose. A comprehensive study of the experimental factors affecting the determination of specific surface was made including the following variables: pressure differential, method of determining rate of flow of air, porosity, cross-sectional area of sample tube, and length of sample tube.

The theory of the permeability method and details of experiments conducted to test its validity have been discussed by Carman (1937, 1938, 1939) and lately reviewed in detail by Sullivan and Hertel (1942).

Experimentally the method determines the rate of flow of a fluid through a cylindrical plug of a porous material.

The specific surface can be expressed by the equation:

$$S_w = \frac{14}{d_a}\sqrt{\frac{A\Delta Pt}{Q\eta L}\cdot\frac{\epsilon^3}{(1-\epsilon)^2}} \qquad (1)$$

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where

S_w = specific surface of the powder in sq. cm. per gram
d_s = density of the powder
A = cross-sectional area of the powder in sq. cm.
L = thickness of the powder in cm.
ΔP = pressure difference driving the fluid through the medium in grams per sq. cm.
Q = volume of air in ml. flowing through the powder in time t, expressed in seconds
η = viscosity of the fluid in poises
ϵ = porosity $= 1 - \frac{W}{d_s A L}$
W = weight of powder in grams.

Carman has shown for certain materials that for the same powder at different porosities the porosity function, $\epsilon^3/(1-\epsilon)^2$, is reasonably accurate over a fairly wide range of porosities. To see if this were true in the case of a single wheat flour a number of determinations were made at different porosities. Rearranging equation (1) into the following form:

$$\frac{\epsilon^3}{(1-\epsilon)^2} = \frac{S_w^2}{K^2} \cdot \frac{\eta}{\Delta P t} \tag{1a}$$

where

$$K = \frac{14}{d_s}\sqrt{\frac{A}{QL}}$$

and by plotting the porosity function $\epsilon^3/(1-\epsilon)^2$ vs. $\eta/\Delta P t$ a straight line should result passing through the origin with slope S_w^2/K^2.

Results from the experiments on wheat flour have indicated that equation (1a) is not correct in its present form.

Apparatus for Determining Specific Surface

The essential features of the apparatus developed for determining the specific surface of wheat flour are shown in Figure 1. The apparatus consists of a vacuum source connected to two surge bottles G and regulated by pinch clamp H which establishes a pressure differentially measured by kerosene manometer F across the cylindrical sample tube A. Ten machined brass sample tubes ranging in diameter from 0.4 cm. to 1.2 cm., and in length from 2.0 cm. to 4.0 cm., have been used in the apparatus. The volume of each tube was determined by filling with mercury and weighing. The length of the tube was determined by use of a micrometer. By dividing the volume by the length, the average cross-sectional area of the tube was calculated.

Various methods have been described in the literature to measure the pressure head across the sample plug and to determine the volume of air passing through the plug in a given length of time. One of the simplest forms of apparatus described employs an open end manometer to measure the pressure differential, and a pipet dipping into a flask containing water to measure the volume of air. Use of such an apparatus was found to be unsatisfactory because the level of water drawn into the pipet acted as an additional arm of the manometer, and the

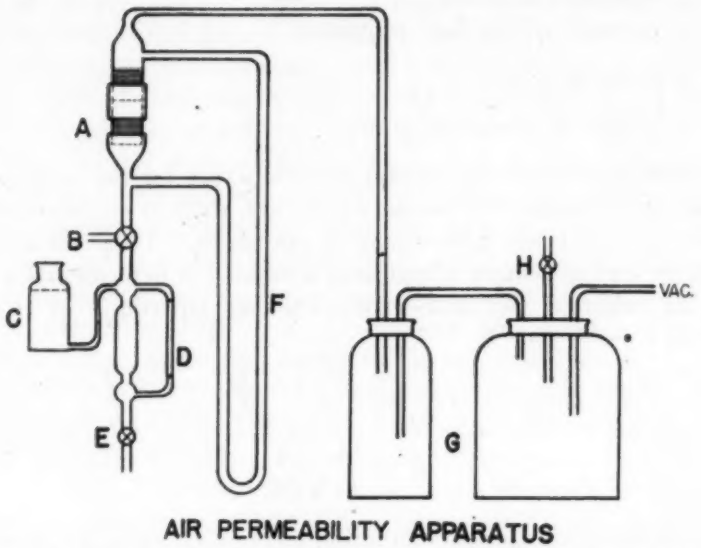

Fig. 1. Air permeability apparatus. (A) sample tube, (B) three-way stopcock, (C) kerosene reservoir, (D) volume measurement device, (E) two-way stopcock, (F) kerosene manometer, (G) surge bottles, (H) vacuum regulator.

pressure determination was consequently dependent upon the volume measurement. In order to avoid this difficulty a direct-connecting manometer F, Figure 1, was used. The volume of air was determined by apparatus BCDE. With the three-way stopcock B open to the air, a pressure differential is established across A. After steady flow has been attained, usually in three minutes, B is closed and air from system D is drawn through A. As the pressure in D is lowered, kerosene from C flows into D replacing the air. The kerosene level in C is adjusted so that a lowering of pressure approximating 1 mm. of kerosene in D causes the liquid in C to flow into chamber D. Thus the pressure measurement by manometer F is not affected appreciably by the volume measurement. The rate of flow of air through A is determined by measuring the time that it takes for the kerosene to fill the space in container D, defined by two etch marks in a capillary

side arm. This volume in the apparatus used is 4.977 ml. The capillary side arm is used because of the greater ease of reading the kerosene meniscus. When the determination is complete the kerosene is drained from D by stopcock E and returned to reservoir C.

To achieve uniform porosity throughout the length of a sample tube a special tamping apparatus, Figure 2, is used. A sample tube

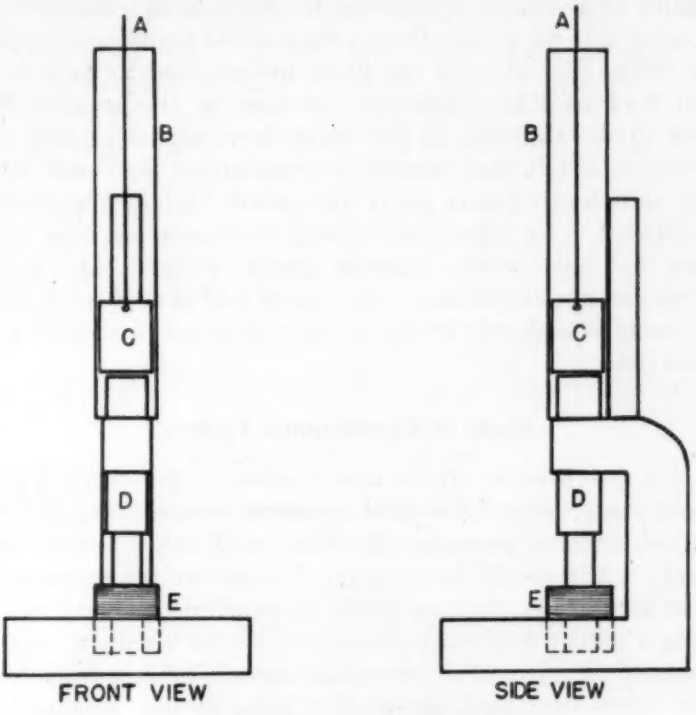

Fig. 2. Tamping apparatus. (A) wire handle, (B) glass guide tube, (C) bucket containing lead shot, (D) steel plunger, (E) machined sample tube.

is screwed into the bottom plate E and a plunger of outside diameter 0.002 inch less than the inside diameter of the sample tube is placed in the tamping apparatus. A small amount of flour, 0.08–0.10 g., is added to the tube and a weighted bucket C is dropped a given distance through glass tube B, hitting the plunger D. By changing the weight of C, the distance through which it falls, or the number of times it drops, a tube may be packed to any desired uniform porosity. The weight of the flour is determined by weighing the sample tube before and after filling.

The sample tube is then placed in the permeability apparatus and a constant pressure differential established. After three minutes the

volume measurement is made and the actual pressure differential is read at the same time. If desired, the rate of flow of air can be determined at several different pressures. The temperature of the room is observed after each determination so that the correct density of kerosene and also viscosity of air may be read from appropriate tables or curves.

Density of a flour is determined by methods described by Bauer (1945) using a 25 ml. pycnometer, xylene as the liquid, and a temperature of 25°C. For most of the flours investigated to date d_s varies between 1.42 to 1.45 grams/ml. Because of the small difference observed in the densities of the flours investigated to date in this laboratory, it is felt that density determinations need only be made on those samples that have either abnormally high or abnormally low water contents. No appreciable change in density has been observed in flours that have widely different specific surfaces, i.e., density is not a function of particle size. A value of 1.45 is used for d_s for most routine samples and only in special cases is a separate density determination made.

Study of Experimental Factors

To test any possible effects due to different pressures, a series of tests was made using differential pressures ranging from 0.2 cm. to 100 cm. of kerosene pressure. By holding all other factors constant the product ΔPt should be constant throughout the pressure range. This was found to be the case within an experimental error of 1%.

Using a patent flour made from hard winter wheat, a comprehensive study of the effect of the size of the sample tubes was made. Ten different tubes were used, the smallest being 20 mm. long and 4 mm. diameter (designated as Tube 20–4), and the largest 40 mm. long and 12 mm. diameter (Tube 40–12). Calculations based on equation (1) gave a value of the specific surface of approximately 2,000 sq. cm. However, there was a standard error of 10% of a single determination from the mean among the 50 determinations made using the 10 different sample tubes.

By adding a correction constant, b, to equation (1a) an equation results which satisfies the data to a better degree:

$$\frac{\epsilon^3}{(1-\epsilon)^2} = \frac{S_w^2}{K^2} \cdot \frac{\eta}{\Delta Pt} + b. \qquad (2)$$

Figure 3 shows the data for Tube 40–12 plotted according to equation (2). All 10 tubes gave similar plots, indicating that the porosity function $\epsilon^3/(1-\epsilon)^2$ is not a sole function of porosity, ϵ. The

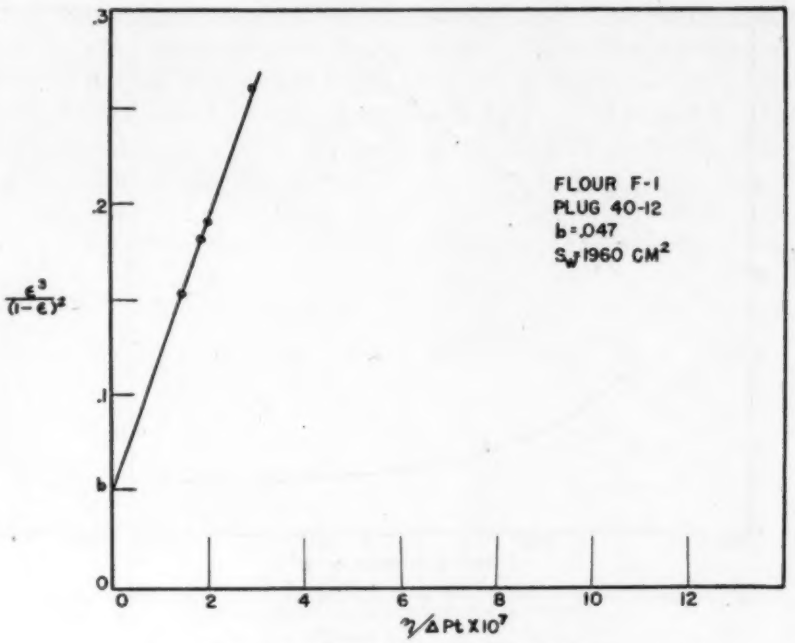

Fig. 3. Experimental plot using equation (2).

values of S_w for the different tubes along with the values of the intercept, b, are presented in Table I. The standard error in S_w is only 2.6%.

TABLE I

SPECIFIC SURFACE OF PATENT FLOUR CALCULATED BY USE OF EQUATION (2)[1]

Tube	S_w cm.^{2}	b
20–4	2120	0.030
20–5	2020	0.036
20–6	1950	0.035
20–8	1990	0.044
20–10	2020	0.040
20–12	2080	0.034
30–6	2020	0.039
30–12	2010	0.037
40–6	2170	0.036
40–12	1960	0.047
Average	2035	0.038

[1] $\frac{\epsilon^3}{(1-\epsilon)^2} = \frac{S_w^2}{K^2} \cdot \frac{\eta}{\Delta P t} + b.$

The values of S_w calculated from (2) are dependent upon the dimensions of the sample tube as is shown in Figure 4. A minimum in the value of S_w was found for a sample tube having a cross-sectional area of 0.40 cm. and length of 2 cm., or a value of $\sqrt{A/L}$ of approxi-

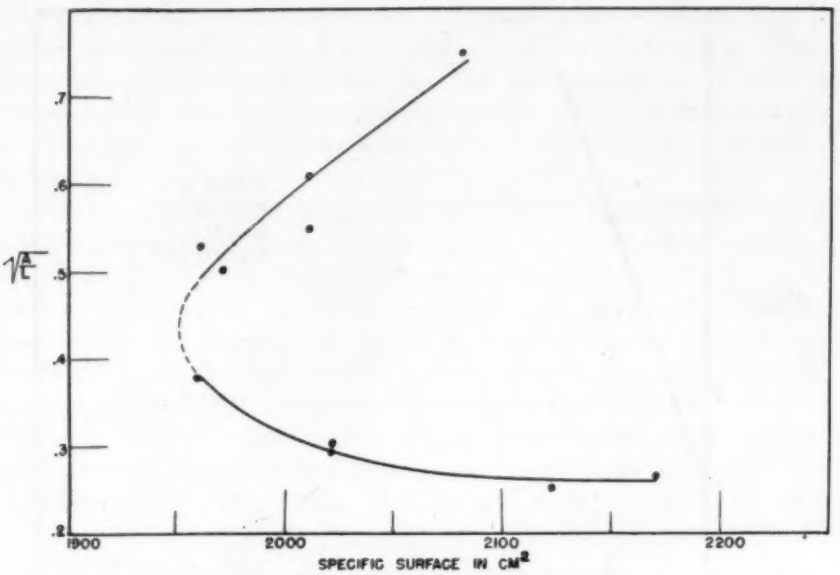

Fig. 4. Specific surface of patent flour (F-1) as a function of the dimensions of sample tube based on calculations from equation (2).

mately 0.45. This indicates that S_w is dependent upon some function involving A and L. Accordingly, equation (2) was modified so that all terms involving A and L were transposed to the left side, giving:

$$\frac{A}{L} \cdot \frac{\epsilon^3}{(1-\epsilon)^2} = S_w^2 \cdot \frac{d_s^2 Q\eta}{14^2 \Delta P t} + C, \qquad (3)$$

where C is a correction term.

By plotting $A\epsilon^3/L(1-\epsilon)^2$ vs. $d_s^2 Q\eta/14^2\Delta P t$ the slope will be S_w^2 and intercept C.

TABLE II

SPECIFIC SURFACE OF PATENT FLOUR CALCULATED BY USE OF EQUATION (3)[1]

Tube	$\sqrt{\frac{A}{L}}$	S_w cm.²	C
20–4	0.253	2170	0.0016
40–6	0.264	2180	0.0018
20–5	0.297	2010	0.0032
30–6	0.305	2020	0.0038
20–6	0.373	1980	0.0047
20–8	0.504	1960	0.0115
40–12	0.530	1980	0.0138
30–12	0.612	1960	0.0175
20–10	0.616	1950	0.0200
20–12	0.745	1950	0.0340
Average of tubes 20–6 to 20–12		1963	

[1] $\frac{A}{L} \cdot \frac{\epsilon^3}{(1-\epsilon)^2} = S_w^2 \cdot \frac{d_s^2 Q\eta}{14^2 \Delta P t} + C.$

Figure 5 shows the data for Tube 40–12 plotted according to equation (3). All 10 tubes gave similar plots. The corresponding values of S_w and C for the various tubes are given in Table II. The values of S_w decrease with increasing values of $\sqrt{A/L}$, see Figure 6, until a value of $\sqrt{A/L}$ of .373 is reached, after which S_w is independent of $\sqrt{A/L}$ within $\pm 1\%$ variance.

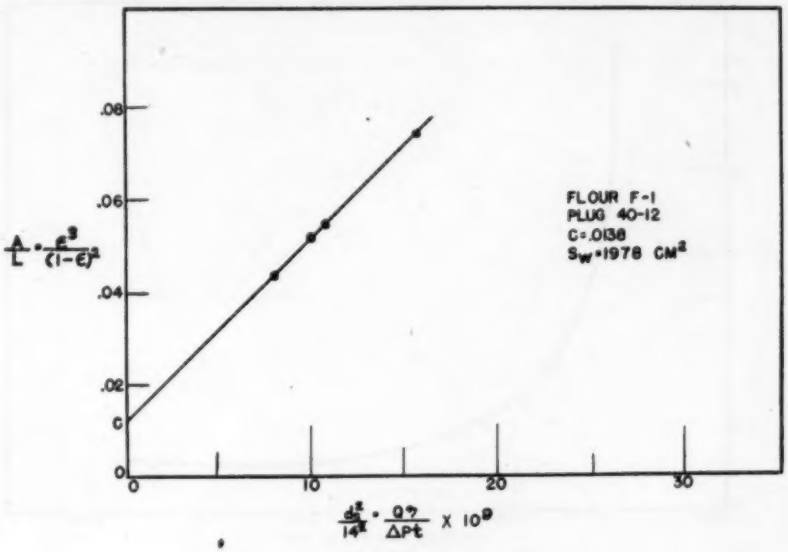

Fig. 5. Experimental plot using equation (3).

Considering the results presented in Figure 6, it is postulated that a small layer of flour adjacent to the walls of the sample tube is inactive and probably very little air will flow through this layer. If this is true, sample tubes of small cross-sectional area or of extreme length would give values of S_w that were too high. However, if the cross-sectional area of the sample tube were increased or the length decreased, the percentage effect of an inactive layer would gradually decrease. This postulate needs to be verified by studies of other materials and a comprehensive review of the dynamics of fluid flow through powder beds.

Experimental reproducible results with a standard error of 0.5% can be obtained in the case of wheat flour by using equation (3) for the calculation of S_w, provided that the ratio of $\sqrt{A/L}$ of the sample plug is greater than 0.4. At least two determinations are necessary by this method to find S_w. In order to evaluate S_w by one determination the function of S_w vs. C must be known. This function was determined for Tube 20–8 and is plotted in Figure 7. The value of

C for sample Tube 20–8 increased as the specific surface of a flour decreased. To make a corrected calculation of S_w from one experimental determination, an approximate S_w is found using equation (1) and by use of Figure 7 the value of the C term is estimated. Then substituting this value of C into equation (3) a corrected value of S_w may be obtained.

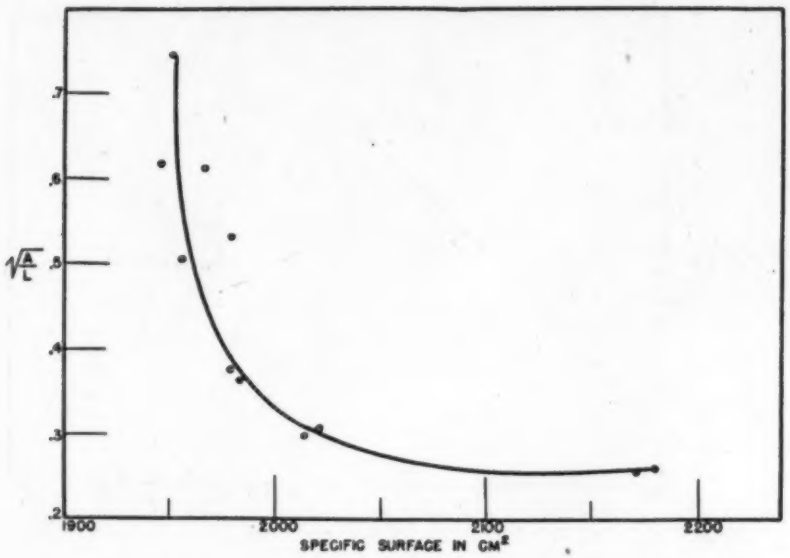

Fig. 6. Specific surface of patent flour (F-1) as a function of the dimensions of sample tube based on calculations from equation (3).

Since no previous values of the specific surface of flour have been presented in the literature, a calculation of the particle size diameter has been made assuming that the flour particles are smooth spheres. The relation between S_w and D, the corresponding diameter of equivalent smooth spheres, is:

$$D = \frac{6}{S_w d_s}. \tag{4}$$

Comparison of equivalent particle diameters with the results of sedimentation experiments of Kent-Jones, *et al.* (1939, 1941) and Hildebrand, Ferrari, Borchardt, and Anker (1942) indicates that the equivalent diameters obtained from surface area measurements are smaller than those obtained either by sedimentation methods or by microscopic observations. This discrepancy is undoubtedly due to the fact that both the sedimentation and microscopic values do not take into account the irregular shape of the flour particles. The jagged appearance of flour particles is readily seen microscopically and this

irregularity of the surface will increase the surface area. Consequently any equivalent diameter calculation based on equation (4) will be smaller in value. This conclusion is borne out by the data of Emmett (1942) on zinc oxide pigments. He found that the values of equivalent diameter determined by air permeability and adsorption methods agreed very closely, but diameters obtained by direct microscopic count were twice as large. Practically, the surface area calculations are more significant than equivalent diameter calculations, but

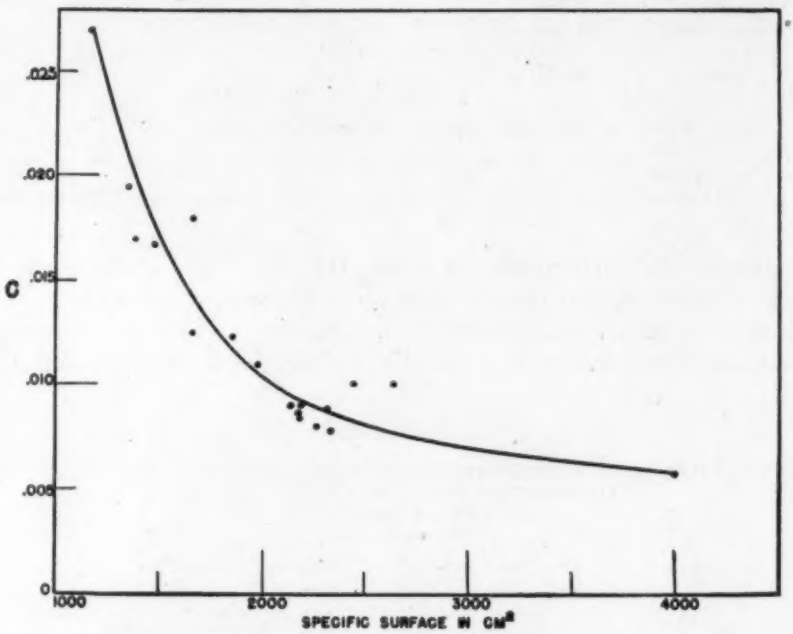

Fig. 7. Variation of correction term C with specific surface. Sample tube 20–8.

for comparison with previous results equivalent diameter calculations are presented in Tables III, IV, and V.

Results and Discussion

Determinations of the specific surface of fractions of Patent flour held on wire sieves and on silk bolting cloths have been made as well as determinations of the specific surface of 45 mill streams from hard red winter wheat. Specific surface determinations of the fractions of flour held on various ASTM wire sieves are presented in Table III. The S_w value for the overs of ASTM 100 mesh is slightly greater than the value for ASTM 140 mesh. This is probably due either to the short length of time of the shaking process or to agglomeration of some of the flour particles. The total surface area for each flour fraction

TABLE III

SPECIFIC SURFACE DETERMINATIONS ON FRACTIONS OF PATENT FLOUR OBTAINED BY USING ASTM WIRE SIEVES (IN RO-TAP SHAKER FOR 10 MINUTES)

ASTM sieves	Weight, grams	Specific surface, S_w in cm.^{2}	Diameter, D, in microns	Total surface area, grams $\times S_w$
Over 80	0.136	—	—	—
Over 100	10.958	1380	29.9	15120
Over 140	8.239	1350	30.6	11100
Over 180	5.339	1660	24.9	8850
Over 300	23.400	2440	16.85	57300
*Under 300	0.459	—	—	—
Total	48.531			92370

Total exclusive of "over 80" and "under 300" = 47.936.

$S_w = \frac{92370}{47.936} = 1{,}930$ cm.^{2}, the average specific surface from above sieve analysis.

$S_w = 1{,}960$ cm.^{2}, the average specific surface as determined on original flour.

is given in the fifth column of Table III. The sum of these surface areas divided by the total weight gives a value of 1,930 cm.^{2}. The value of S_w of the original flour was 1,960 cm.^{2}. This agreement is a good substantiation of the experimental technique of determining S_w.

TABLE IV

SPECIFIC SURFACE DETERMINATIONS ON FRACTIONS OF PATENT FLOUR OBTAINED BY USING SILK BOLTING CLOTHS 15 MIN. (ROTARY MOTION) (50 GRAMS TOTAL)

Silk	Weight, grams	Specific surface, S_w in cm.^{2}	Diameter, D, in microns	Total surface area, grams $\times S_w$
Over 9XX	0.334	—	—	—
Over 12XX	2.016	1480	28.0	2975
Over 15XX	19.028	1160	35.5	22190
Over 20 std.	11.790	2140	19.3	25180
Over 25 std.	13.485	2640	15.6	35600
Under 25 std.	2.888	4000	10.3	11550
Total	49.541			97495

Total exclusive of 9XX = 49.237.

$S_w = \frac{97495}{49.237} = 1{,}970$ cm.^{2}, the average specific surface from above silk analysis.

$S_w = 1{,}960$ cm.^{2}, the average specific surface as determined on original flour.

The same type of results presented in Table IV were obtained using silk bolting cloths. The flour over 12 xx silk had a higher value of S_w than the fraction held on 15xx silk, indicating either too short a shaking time or agglomeration. The weighted surface area calculated

from these various samples agreed within 1% of the value of S_w determined on the original flour.

The results of the specific surface studies on 45 mill streams are presented in Table V. The flour from the break rolls shows a considerable variation in specific surface. The flour from 3 Break N has a low specific surface which correlates with the low diastatic activity of that particular mill stream. The Cuts stream has the lowest

TABLE V

SPECIFIC SURFACE OF MILL STREAMS

Stream	Moisture %	Ash %	Protein %	Diastatic activity,[1] mg. maltose	Specific surface, S_w in cm.^{2}	Diameter, D, in microns
1 Break N	16.6	0.57	11.8	143	2130	19.3
S	16.5	0.57	11.7	138	2000	20.6
2 Break N	16.3	0.50	12.0	138	1960	21.0
M	16.4	0.51	12.2	128	1940	21.2
S	16.5	0.52	12.2	138	2043	20.2
3 Break N	16.4	0.50	13.1	98	1670	24.6
S	16.2	0.51	13.2	118	2142	19.2
4 Break N	15.7	0.57	14.3	133	2190	18.8
S	15.6	0.57	14.4	133	2178	18.9
5 Break N	15.4	0.69	15.5	133	2230	18.5
S	15.1	0.69	15.5	133	2170	19.0
1 Siz N	15.0	0.47	10.8	304	2240	18.4
S	15.0	0.47	10.7	323	2185	18.8
2	14.6	0.50	11.1	308	2500	16.5
B	15.3	0.58	11.5	158	1975	20.9
Germ	14.4	0.65	11.1	287	2720	15.2
1 Tail	14.1	0.58	11.0	355	2835	14.5
2	13.7	0.59	10.8	388	2690	15.3
3	12.7	0.91	12.8	298	2902	14.2
1 Low Grade	13.2	0.62	12.0	269	2270	18.2
2	12.9	0.56	11.6	292	2440	16.9
3	12.3	0.71	11.8	317	2340	17.6
Bran and Shorts N	15.0	1.03	15.2	178	2450	16.8
S	15.0	1.03	15.2	178	2550	16.2
Patent	14.6	0.42	11.0	294	2162	19.0
Clear	14.3	0.75	13.1	365	2380	17.3
1 Mids N	14.7	0.38	10.8	306	2332	17.7
E	14.7	0.38	10.8	312	2315	17.8
S	14.3	0.37	10.7	306	2438	16.9
2 Mids N	14.4	0.38	10.6	269	2180	18.9
M	14.6	0.38	10.7	269	2260	18.2
S	14.3	0.38	10.6	275	2190	18.8
3 Mids N	14.4	0.41	10.7	253	2185	18.8
S	14.3	0.39	10.7	248	1855	22.2
4 Mids N	13.5	0.39	10.6	275	2217	18.6
S	13.5	0.39	10.6	275	1982	20.8
5 Mids	13.6	0.44	11.2	253	1762	23.4
6 Mids	13.3	0.46	11.1	296	2260	18.2
7 Mids	13.3	0.48	11.5	275	2222	18.5
Cuts	16.2	0.45	12.2	103	1435	28.8

[1] Total maltose after diastasis for one hour. Method 20.61 of Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists.

specific surface of any of the flours tested. The Germ, Tailings, Low Grades, Bran, and Shorts streams have relatively high specific surfaces. As indicated by these results the various streams that are blended to produce Patent flour vary as much as 20% in specific surface values.

Summary

A rapid method for the determination of the specific surface of wheat flours by the air permeability is presented. A critical study of several experimental factors influencing the reproducibility of results has been made. The size of the sample tube is critical, the best results being obtained with tubes having dimensions such that the square root of the ratio of cross-sectional area to length is greater than 0.4. It has been found necessary to revise the air permeability theory in order to reduce the dependency of specific surface on the porosity function.

Patent flours, made from hard winter wheat, have been found to have a specific surface of about 2,000 cm.^{2}. The specific surface of various sieve fractions of such a flour indicate that it is composed of material having a specific surface of 1,200 to 4,000 cm.^{2}. Determinations on 45 mill streams indicate that the streams blended to produce Patent flour vary as much as 20% in specific surface values.

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CHARACTERIZATION OF WHEAT GLUTEN. I. PROTEIN-LIPID COMPLEX FORMATION DURING DOUGHING OF FLOURS. LIPOPROTEIN NATURE OF THE GLUTENIN FRACTION

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(Presented at the Annual Meeting, May 1947; received for publication July 14, 1947)

Several investigators have observed that gluten, as it is ordinarily obtained from flour, contains 5 to 10% lipids (Dill, 1925), and that only a small fraction of these can be extracted with petroleum or ethyl ether (Dill, 1925; Fisher and Halton, 1933; Blish, 1936; Sullivan, 1940; and others). McCaig and McCalla (1941) suggested that this protein-lipid complex may be formed when the dough is made. The experiments to be described here confirm this concept in part. In addition, it has been possible to demonstrate that mere wetting of flour causes binding of a considerable part of the lipids. Furthermore, fractionation studies with gluten have shown that most of the lipid is associated with the "glutenin," rather than the gliadin, portion. These findings suggest that glutenin, as it occurs in gluten, but not necessarily in the wheat grain, should be considered a *lipoprotein*.

Materials and Methods

The flour used in most of the experiments was an unbleached bakers' flour, containing 10.5% moisture and, on a dry basis, 0.4% ash and 15.3% protein (nitrogen × 5.7).

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The extractability of the lipids from flour, dried doughs, and glutens was determined on samples which had been thoroughly dried in a vacuum oven at 60°. Total lipids were determined by extraction with absolute alcohol in the Soxhlet apparatus, followed by evaporation of the alcohol and resolution of the lipid fraction in chloroform. Only the chloroform-soluble fraction was weighed. That such extraction was essentially complete was demonstrated as follows: A weighed portion of gluten was hydrolyzed with 10% potassium hydroxide, acidified, and extracted with ether. The ether-soluble fraction represented the fatty acids plus the unsaponifiable fraction of the gluten lipids. Its weight was in good agreement with a similar fraction obtained from the alcohol extract of the same gluten sample (see also Tucker, 1946).

Ether-soluble fractions were obtained (1) by Soxhlet extraction, (2) by centrifugation and decantation of several portions of solvent, and (3) by percolation of the solvent through a column of the material. Results obtained by the latter two methods were in agreement and gave more consistent, but slightly lower, results than did Soxhlet extraction.

In some cases flours were worked up into doughs by hand. In others, doughs were prepared as follows: 15 g of the flour and 15 ml of water were placed in a Waring Blendor bowl. The motor of the Blendor was connected to a varying resistance so that it could be run at a very low speed. Doughs of uniform consistency were readily obtained in very short mixing times.

Doughs, glutens, and protein solutions were dried by lyophilization, that is, they were frozen and the water was removed by sublimation in a high vacuum. The dried products had a porous structure and were readily reduced to fine powders, in contrast to the hornlike materials so often obtained when gluten is dried by other techniques. This particular method of handling materials is perhaps the most important advance over previous investigations. The fineness of the dried powders lent increased confidence in the validity of extraction data.

Effect of Wetting and Doughing on the Solubility of Flour Lipids in Ether

Approximately 70% of the total lipids of the flour could be extracted with ether. Similar data have been reported by other investigators (cf. Sinclair and McCalla, 1937; Sullivan and Howe, 1938; Barton-Wright, 1938; Tucker, 1946).

A sample of the flour was wet with water in such manner as to avoid any mechanical doughing action. The flour was added, in small amounts at a time, to an excess of water that was kept stirred. No

clumps formed. The mixture was quickly frozen and dried. Only 40% of the lipids could now be ether-extracted.

An attempt was made to determine the least amount of water that would cause such a loss in solubility. A sample of the flour was placed in a closed vessel over water (100% relative humidity). Toluene vapor was present to prevent mold or bacterial growth. At the end of three weeks, the sample was found to contain 20% moisture. It was then dried (vacuum oven, 60°) and extracted with ether. As with the original flour, 70% of the total lipids were extractable. It may be concluded that the very high moisture contents that occasionally occur under adverse storage conditions would probably not cause additional gluten-lipid combination.[2]

TABLE I

EXTRACTABILITY OF LIPIDS OF FLOUR

	Of flour	Of total lipids
	%	%
Alcohol-extractable fraction	1.45	100
Ether-extractable fraction (EEF)	1.0	70
EEF—flour stored at 20% H_2O content	1.0	70
EEF—flour brought to 30% H_2O content	0.57	39
EEF—flour wet, then dried	0.55	38
EEF—flour doughed, then dried	0.09	6

Another sample of the flour was brought to 30% moisture content in the following manner: The calculated amount of powdered ice was thoroughly mixed with a previously cooled sample of the flour at −34.4°C. After one month at −9.4°C, during which time most of the ice sublimed into the flour, the mixture was brought to room temperature. It then formed a very stiff dough. The dough was carefully broken into small pieces and dried by lyophilization. In this sample, only 40% of the total lipids were extractable by ether. These preliminary observations indicate that the critical moisture level for the conbination, or "binding," of lipids is between 20 and 30%, and that, if mechanical doughing is avoided, an approximately equal amount of the flour lipid (30%) becomes bound whether a minimum amount or an excess of water is added.

Portions of the flour were made into doughs by the Waring Blendor technique, then dispersed in excess water or dilute acetic acid. The dispersions were frozen and dried. Now only 6% of the lipids could be extracted with ether.

These observations (summarized in Table I) show that approximately 30% of the lipids of the particular wheat flour used are bound,

[2] The free lipid to bound lipid ratio of 2:1 is typical for high protein *flours*. It does not necessarily reflect the ratio in the wheat endosperm. The results of the experiments described in this report suggest the possibility that some changes in the state of the lipid fraction might occur during storage or milling operations.

that is, they cannot be extracted with ether. When the flour is wet with water, another 30% becomes bound, and when it is kneaded into a dough, a third 30% becomes bound. The bonds holding the lipid in combination are formed in the presence of water, and broken in the presence of alcohol.

Capacity of Flour to Bind Lipids

Samples of flour containing excess flour lipids were prepared as follows: A petroleum ether extract of the flour was added in varying quantities to separate aliquots of the unextracted flour. After most of the solvent had evaporated, the last traces were removed in a vacuum oven (60°). These samples, which now contained up to 8.5% total lipids, were made into doughs by the Waring Blendor technique, dispersed in excess water, frozen, and dried. The dried doughs were powdered, then extracted with ether by the centrifugation and decantation method. The results (Table II) demonstrate clearly that an

TABLE II

BINDING OF EXCESS LIPID BY FLOUR DURING DOUGHING

Sample	Total lipid in dried dough	Lipid extractable by ether	Bound lipid	Bound lipid as percent of total lipid
	%	%	%	%
1[1]	1.45	0.10	1.35	93
2	2.30	0.25	2.05	89
3	3.14	0.34	2.80	89
4	4.82	0.64	4.18	85
5	8.42	4.31	4.09	49

[1] No added lipids.

amount of lipid up to about three times that in the original flour can be bound so that it is no longer available for ether extraction, but that this amount apparently approaches the capacity of the flour.

Preferential Binding of Phospholipids

It has been assumed that the phosphorus content of the lipid fractions represents the phospholipids present. The results of phosphorus analyses obtained during the experiment just described are shown in Table III. They indicate that even when four times the original amount of phospholipid is present, only 3% can be extracted by ether, in contrast to the half of the total lipids so removable.

In a separate experiment, it was found that the ether extract of a flour that had been wetted and then dried contained 30% of the total lipid but only 4% of the total lipid-phosphorus. The ether extract of the original flour contained 70% of the total lipid and 30% of the lipid-

TABLE III

PREFERENTIAL BINDING OF PHOSPHOLIPID

Sample	Lipid phosphorus present in dried dough	Phosphorus extracted with ether as percent of total phosphorus	Bound lipid phosphorus	Bound lipid as percent of total lipid
	%	%	%	%
1	0.007	0	100.0	93
2	0.010	0	100.0	89
3	0.013	0.4	99.6	89
4	0.018	1.7	98.3	85
5	0.030	2.8	97.2	49

phosphorus. Thus, during the wetting procedure, half of the ether-soluble lipids but almost 90% of the ether-soluble phosphorus became bound. Pending confirmation in detail, these observations suggest that phospholipids are bound preferentially, compared to other constituents of the flour lipids.

The marked differences in extractability between total lipid and lipid-phosphorus, before and after doughing, may be taken as additional evidence that the changes observed are real and not due to mechanical interference with the extraction procedure.

Participation of Gluten

The experiments described so far have dealt with changes in the amounts of lipids extractable by ether from *flours*. The role of the flour proteins in these phenomena remains to be elaborated.

Fisher and Halton (1933) observed that considerable amounts of lipids added to flours may be retained in the gluten washed from them. Sullivan (1940) states, "When gluten is washed out from flour it contains over half of the lipids of the flour. The gluten must first be treated with alcohol in order to liberate the lipids, since only a trace can be removed by direct extraction with ethyl ether or petroleum ether." (Cf. also Dill, 1925.) Our experiments confirm such observations. With the flour at our disposal, approximately 70% of the total lipids could be accounted for in the gluten fraction after thorough washing. (Fisher and Halton, 1933, record 70%.) The dried gluten contained 7.4% total lipids, approximately one-third of which were extractable with ether. The validity of the extraction data was demonstrated as follows: One sample was dried directly from the frozen state; another was dispersed completely in dilute acetic acid, then frozen and dried. The results of extraction procedures with these samples were in agreement. These results and the observations of previous workers indicate that the binding of lipid in flour is largely a function of the gluten.

In order to determine which of its components is involved in this reaction, we have fractionated gluten by several techniques introduced by previous investigators (see Blish and Sandstedt, 1929; Spencer and McCalla, 1938; Blish, 1936). In each case the bulk of the lipids was found to follow those fractions usually designated by the term "glutenin."

One fractionation was accomplished briefly as follows: Gluten was dispersed in dilute acetic acid, as first suggested by Blish and Sandstedt (1926). Excess starch and a small amount of insoluble protein were removed by passing the solution through a Sharples centrifuge. Proteolytic enzymes were destroyed by a brief heat-treatment (Olcott *et al.*, 1943).[3] The glutenin fraction was separated by adjusting the solution to pH 5.0, and purified by several resuspensions in solutions of pH 5 to 6, followed by centrifugations. The combined supernatant solutions were brought to pH 6.8 and the precipitate separated. This precipitate was stirred with water containing acetic acid to pH 5.5, then again centrifuged. The insoluble fraction is referred to as the "middle fraction" in Table IV. The soluble portion was considered to

TABLE IV

DISTRIBUTION OF LIPIDS IN GLUTEN FRACTIONS

	Fraction recovered	Lipid content	Lipid as percent of total lipid
	%	%	%
Glutenin	46	20.0	81.5
Middle fraction	13	11.2	13.0
Gliadin	41	1.5	5.5

be mostly gliadin. All preparations were dialyzed and dried by lyophilization. The results of extraction experiments are shown in Table IV. Very little of the total lipids were extractable with ether. Alcohol disrupted the bonds that held the lipid to the protein (cf. Blish, 1936; Sullivan, 1940). Such properties are similar to those of many protein-lipid complexes now recognized as occurring widely in nature (see Chargaff, 1944). They suggest that glutenin, as it occurs in wheat gluten, should be considered a lipoprotein.

Several years ago, Blish (1936) called attention to the presence of a protein-lipid complex in gluten, and suggested that the portion of wheat gluten other than gliadin was composed of a mixture of glutenin and the complex. Small fractions containing amounts of lipid ranging up to 44% were isolated. So far we have not obtained fractions containing more than 15–20% lipids (from normal flour), nor have we been

[3] Enzyme activity is destroyed by exposure of the gluten solution in dilute acetic acid to a temperature of 90°–100° for as short a time as 30 seconds (Olcott and Sapirstein, unpublished experiments).

able to differentiate between glutenin and the lipoprotein complex. Until this is accomplished, it appears preferable to identify glutenin with the lipoprotein complex.

It is of interest that Dill in 1925 described a preparation of glutenin in which nonaqueous solvents had been avoided. The product contained 11% total lipids, and only 3% ether-extractable lipids. The significance of his observation has escaped attention.

Discussion

The recognition that the lipid and the protein of wheat flour form a lipoprotein complex *during* the wetting and doughing of flour may help in the unraveling of some of the, as yet, little understood aspects of bread technology.

Some of the difficulties hitherto encountered in attempts to isolate and characterize the components of gluten may be attributed to the unrecognized lipoprotein nature of glutenin. Many investigators have agreed on the properties of gliadin (Larmour and Sallans, 1932; Blish, 1945), a protein that is apparently not involved in lipid binding. Few have agreed on the properties of glutenin. The use of alcohol or alkali in its preparation disrupted the lipoprotein complex and irreversibly changed its properties (see Sinclair and McCalla, 1937).

The existence of *glutenin* as an entity is debatable, and therefore even the use of the term is of doubtful propriety. Nevertheless, it is serviceable as a name for the "nongliadin-like fraction" of wheat gluten. For future use we suggest "lipoglutenin" to indicate this protein moiety as it exists in gluten, and "glutenin" for the same fraction from which the lipids have been extracted. These designations conform to the terminology suggested by Chargaff (1942, 1944) for the egg yolk proteins, "lipovitellin" and "vitellin."

Experiments along the lines indicated in this paper are now being applied to other types of flours, and the results will be presented in detail in subsequent publications.

Summary

A high-protein patent flour contained 1.5% total lipids, 70% of which were extractable with ether. The flour was mixed with water with a minimum of doughing, then dried by lyophilization. Only 40% of the lipids were now extractable with this solvent. After the flour was kneaded into a dough and dried, less than 10% of the lipids could be extracted.

The capacity of the flour to "bind" lipids during wetting and doughing was ascertained by determining the extractability of added flour lipids. At least three times the amount of lipid normally present

could be bound by the doughing procedure. Phospholipids were bound preferentially.

Most of the lipid bound was associated with the gluten, rather than with the nonprotein, constituents of flour; and, when gluten was fractionated, the lipid was found to be bound to the "glutenin," rather than to the gliadin fractions.

Glutenin fractions containing up to 20% lipids have been obtained. It is proposed to call the nongliadin fraction of gluten, "lipoglutenin." The term "glutenin" should be reserved for fat-free preparations.

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RELATION OF SUSCEPTIBLE STARCH, ALPHA-AMYLASE, AND SUGARS ORIGINALLY PRESENT TO FLOUR GASSING POWER

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(Received for publication April 29, 1946)

The correlation between gas production and diastatic activity has been studied by Larmour, Geddes, and Whiteside (1933), Sandstedt and Blish (1934), Graesser (1936), Munz and Bailey (1936), Blish, Sandstedt, and Mecham (1937), Davis (1938), Davis and Tremain (1938), Singh and Bailey (1940), and Hildebrand and Geddes (1940). Some found a high correlation between the two measurements; others found it to be barely significant. Fisher, Halton, and Hines (1938) obtained a significant correlation with the total gas only and concluded that the one-hour maltose test could not be used as a safe substitute for the gas production test. All these observers sought a significant correlation between gas produced and diastatic activity, while making no correction for the effect of the fermentable sugars present originally in the flour.

Blish, Sandstedt, and Astleford (1932) appear to have been the first to realize that the original fermentable sugars present in the flours are of considerable importance in predicting their gassing power. They found that the reducing sugar originally present was low in amount, varied little, and could be disregarded, but that the sucrose content varied from 1.00–1.74% and was sufficient to account for the few discrepancies in the gas production of individual flours as predicted from the diastatic activity. Sandstedt (1934) compared the sucrose and the maltose produced by diastasis with the gas pressure produced during a four-hour period and considered that there was a satisfactory agreement between the results obtained by the two methods.

Davis and Worley (1934) found a linear relationship between the diastatic activity and gassing power of 122 flours and calculated the regression equation and the error of predicting one value from the other. They found that the sucrose content was the major factor accounting for the fact that some samples did not fall very close to the average line of relationship. Eva, Geddes, and Frisell (1937) found, on the other hand, that although the correlation between diastatic activity and gassing power was highly significant, it was not sufficiently high to predict the latter from the former with accuracy. The closest relationship was obtained for the 30-minute period pre-

ceding the critical time. Gas production after the critical time was not closely associated with maltose values based on the customary conditions of diastasis, which suggests that such maltose values are conditioned largely by the quantity of the more diastatically susceptible hexosans (dextrins and ruptured granules) present. Increasing the time or temperature of diastasis would therefore be expected to give a truer index of the ability of a flour to support gas production after the critical period. The results obtained by using optical methods, in which the sucrose content is taken into account, for estimating diastatic activity suggested that, while the correlations were not significantly higher than those involving the maltose value, in a more extended series employing more suitable conditions of diastasis these methods might prove superior for the purpose of estimating flour gassing power.

Davis (1938) found that, although the correlation between the gas-producing ability of flours and their diastatic activity values was highly significant, it was difficult to place the flours in order of their gas-producing capacity. Differences in sucrose content accounted for the greater part of the variation. Shellenberger (1938) pointed out that a fair degree of accuracy may be expected if the diastatic activity is used to predict the gassing rate of a flour after the original sugar has been fermented. Sandstedt and Blish (1938) observed that adjustment of flours by addition of sucrose, so as to give equal dough sugar levels, does not ensure equal rates of gas production throughout fermentation. They attributed the differences to a variable content in the flours of a maltose fermentation activator.

The importance of factors other than the original sucrose content of flours in determining the correlation between autolytic diastatic figures and gassing figures has been investigated by several workers. Sandstedt, Blish, Mecham, and Bode (1937) found that some flours had a higher gassing power than could be accounted for by a knowledge of their maltose figures and their sucrose content, and they ascribed this to the presence of a high content of an enzyme responsible for rendering raw starch available to beta-amylase. Such an enzyme was known to occur in sound wheat flour. Blish, Sandstedt, and Kneen (1938) applied a yeast manometric method to the evaluation of malt supplements and found that the gassing responses of different malts (in the first few hours) fell in the same order as did their alpha-amylase values.

Sandstedt (1938), working with a series of flours also investigated by Davis and Tremain (1938), found a very high correlation between their alpha-amylase values and their gassing and autolytic maltose values. The highest correlation was between alpha-amylase and

gassing power. Two malts were encountered in which the gassing power was low, although the alpha-amylase was high. He suggests that the other factor concerned might be a separate enzyme responsible for raw starch saccharification, and that it registers in this gassing method but not in any of the other methods. Bottomley (1938) did not find a good correlation between diastatic activity and gassing power. The discrepancies between actual gassing power and that expected from a knowledge of the diastatic activity and sucrose content were not uniform.

Kneen and Sandstedt (1941) have given a further explanation of the significance of alpha-amylase in determining gassing power in sugar-deficient doughs. This enzyme was found to be responsible for hydrolyzing raw starch and also, in combination with beta-amylase, it carries hydrolysis much further than does the latter enzyme alone. In 1942 they obtained further evidence of the high correlation between the gassing power response caused by malts and their alpha-amylase activity.

Meredith, Eva, and Anderson (1944) showed that the relationship between gas stimulation by malted wheat flours and their alpha-amylase activity occurs both within and between wheat varieties. Their correlation coefficient is not as high as that reported by Kneen and Sandstedt (1942), who stated that determinations of added gassing power and of alpha-amylase appear to be equally reliable for evaluating malts. Gas production in the corresponding unmalted flours was assumed to be correlated with their alpha-amylase content, although this was not determined in the case of the sound wheat flour. Hildebrand and Geddes (1940) have shown that if care is taken to select the proper levels of diastatic activity and gas production, malt dosage may be estimated from either with equal precision, although the use of diastatic activity is much less convenient.

It seems from the foregoing review that the factors to be considered in estimating the potential gas production of a flour are the fermentable sugars originally present and the amount of raw starch-splitting amylase. No measurement has been made of the importance of variation in the starch substrate as a factor in gas production. A fair degree of satisfaction in the estimation of potential gas production has actually been attained by the use of a knowledge of the sucrose content and the diastatic activity of a flour. It has been shown by Dadswell and Gardner (1947) that at least two factors, the alpha-amylase content and the susceptibility of the starch substrate, are intimately related to the diastatic activity figure of a flour. Thus it is reasonable to postulate that the substrate factor should also be considered in gas production.

By using values which measure each factor separately, a better understanding of the role of each in potential gas production should be possible. The simplest combination is the amount of alpha-amylase as represented by the dextrin formed during autolysis at 62°C, the susceptibility of the starch substrate as measured by the maltose due to beta-amylase, and the amount of sugars originally present in the flour as represented by the total of the sucrose and maltose originally present. Comparison between the gas production as estimated on the basis of the diastatic activity figure together with the quantity of sugars originally present and that calculated on the basis of the individual factors concerned is of interest as a means of judging how satisfactorily gas production can be estimated by either method, and whether any advance has been made in the accuracy of estimation by use of the separate factors.

In order to make further observations on the influence of variations in the substrate on gas production, a series of flours having a uniform amylase and sucrose content but a variable substrate on which the amylase could act and representing at the same time different varieties and places of growth would be useful, but such a series is difficult if not impossible to obtain. However, some information could be gained by substituting a uniform concentration of alpha- and beta-amylase for the variable concentration in a series of natural flours and observing the effect of variations in the susceptible starch and in the sucrose content on the gas production.

Notation

Notations for the various gas production figures reported in this paper are similar to those used, for corresponding determinations of sugars and diastatic activity, by Dadswell and Gardner (1947). Since both sets of notations are used in the present paper, the full list is given below:

R = Reducing sugars originally present in flour.
Z = Sucrose originally present in flour.
T = Total sugars originally present in flour, i.e., $R + Z$.
G_T = Gas production due to total sugars (T).
S = Susceptible starch in flour.
G_S = Gas production due to susceptible starch.
M_β = Maltose formed by excess β-amylase.
G_β = Gas production due to excess β-amylase.
M_α = Maltose formed by excess α-amylase.
G_α = Gas production due to excess α-amylase.
A_ε = Gross autolytic diastatic activity.

G_β = Gas production corresponding to above.
A_n = Net autolytic diastatic activity.
G_n = Gas production corresponding to above.
D = α-amylase in flour by dextrose method.
G_1 = Gas production during first 3 hours.
G_2 = Gas production from end of 3rd to 7th hour.
G_3 = Gas production during first 5 hours.
G_4 = Total gas production.

The determinations were made on natural flours and on a corresponding set of artificial flours (see previous paper). Notations for natural flours are given above; notations for artificial flours are similar but a prime mark is added, e.g., G_T', M_β', G_1', etc.

Materials and Methods

Materials. The preparation of crude alpha- and beta-amylase was carried out as previously described by Dadswell and Gardner (1947). Amylase-free flour for use in gassing experiments was prepared by allowing 10 g. of flour mixed with 6 ml. of 0.25 *N* hydrochloric acid to stand at room temperature for 30 minutes. One ml. of 7% sodium carbonate was then added and the dough was well mixed.

Total sugars originally present in flour (*T*). Sucrose (Z) was estimated according to the method of Sandstedt (1937). The reducing sugars originally present (R) were determined as described by Dadswell and Gardner (1947). The sum of these amounts represent total sugars originally present in the flour (T).

Measurement of gassing power. The total gas pressure in mm. of mercury was measured according to the method of Sandstedt and Blish (1934) in a pressuremeter similar to that described by them. The mean internal capacity of the pressuremeters was 275 ± 1.8 ml.

Two sets of results are available for each sample, those based on the natural flours (i.e., on naturally occurring amylases) and those based on the artificial flours (i.e., on the combined action of 60 mg. of crude alpha-amylase and 60 mg. of crude beta-amylase on the amylase-free flour). For the artificial flours, the dry powdered amylases were added to the amylase-free flour followed by 1 ml. of a 30% suspension of bakers' yeast. Fermentation was carried out as for natural flours in a pressuremeter.

The gas pressure produced has been considered in relation to time under four headings: that produced during the first 3 hours being designated as G_1 for the natural flours and as G_1' for the artificial flours; that produced from the end of the third to the end of the seventh hour as G_2 and G_2'; that produced during the first 5 hours as

G_3 and G_3'; and finally the total gas pressures G_4 and G_4', which were the maximum readings obtained.

The total gas pressure results from the fermentation of the sugars originally present in the flour and of those sugars produced as a result of amylase action. The sugars produced by the amylases, corresponding to the gross diastatic activity and denoted by A_g or A_g', are designated when fermented as the gas pressures G_g and G_g'. These sugars were further divided into those due to beta-amylase (M_β) and those due directly or indirectly to the presence of other amylases (A_n and A_n'). The corresponding gas pressures were therefore designated as G_β, G_n, and G_n'. The experimental details concerned in differentiating the gas produced from these various classes of sugars are given below.

Gas pressure due to sugars originally present in flour (G_T). Ten grams of flour, treated to render it amylase-free, were fermented by 1 ml. of 30% yeast suspension in a pressuremeter. The total pressure developed was designated as G_T, which corresponds to the total sugar originally present (T).

Gas pressure due to susceptible starch. (*1*) Gas due to beta-amylase (G_β). Ten grams of flour, treated to render it amylase-free, were mixed with an excess of crude beta-amylase, in this case 120 mg. The total pressure developed was that due to the fermentation of the sugars originally present in the flour and of the maltose formed as a result of the action of beta-amylase on the susceptible starch. Therefore the total pressure developed minus G_T equals G_β, the pressure due to beta-amylase action. G_β has a fixed value for each flour since it is determined in the presence of an excess of beta-amylase and corresponds to the maltose due to beta-amylase (M_β).

(*2*) Gas due to alpha-amylase (G_α). Ten grams of flour, treated to render it amylase-free, were mixed with 60 mg. of crude alpha-amylase. The total pressure developed was due to the fermentation of the sugars originally present in the flour and of the sugar formed as a result of the action of alpha-amylase. Therefore the total pressure developed minus G_T equals G_α.

Gas pressure due to combined action of amylases and susceptible starch. (*1*) G_g, corresponding to the gross diastatic activity, represents the difference between the pressure due to the total gas produced (G_4) and that due to the fermentable sugars originally present in the flour (G_T), and is therefore the pressure due to fermentation of the sugars produced by the action of the naturally occurring amylases ($G_4 - G_T = G_g$).

G_g' corresponding to A_g', the gross diastatic activity for artificial flours, is equal to G_4' minus G_T.

(*2*) G_n, for natural flours, represents the difference between the pressure due to the total gas produced (G_4) and that due to both the fermentable sugars originally present in the flour (G_T) and those formed by beta-amylase acting alone (G_β), and corresponds to A_n, the net diastatic activity figure for natural flours ($G_4 - G_T - G_\beta = G_n$).

For artificial flour $G_n' = G_4' - G_T - G_\beta$.

Results and Discussion

Flours from the same six varieties of white winter wheat, each grown at three places in Victoria, Australia, as were studied in connection with diastatic activity (Dadswell and Gardner, 1947) were used in a study of gas production. The analytical data are recorded in Tables I and II.

TABLE I

SUCROSE, TOTAL SUGARS, AND GAS CORRESPONDING TO SUGARS AND TO GROSS AND NET DIASTATIC ACTIVITY VALUES FOR NATURAL AND ARTIFICIAL FLOURS

Sample number	Sucrose	Total sugars in original flour	Gas formed from total sugars in original flour	Gas formed by natural flours corresponding to:		Gas formed by amylase-free flours with added amylases			
						Excess beta-amylase	60 mg. crude alpha-amylase	Beta- and alpha-amylase corresponding to:	
				Gross diastatic activity	Net diastatic activity			Gross diastatic activity	Net diastatic activity
	Z	T	G_T	G_g	G_n	G_β	G_α	G_g'	G_n'
	mg.	*mg.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
1	260	290	178	439	231	208	279	701	493
2	166	200	143	958	744	214	292	750	536
3	265	301	227	368	203	165	227	668	503
4	251	295	211	525	340	185	256	617	432
5	220	246	148	299	127	172	261	647	475
6	147	181	145	353	234	119	271	490	371
7	250	288	189	581	467	114	242	526	412
8	169	192	138	181	102	79	173	434	355
9	178	204	136	173	65	108	187	482	374
10	229	267	178	728	628	100	192	450	350
11	220	263	195	424	340	84	158	434	350
12	159	189	107	282	161	121	197	423	302
13	217	264	203	211	139	72	155	440	368
14	186	216	155	237	162	75	170	426	351
15	229	269	194	289	191	98	155	473	375
16	139	165	123	134	56	78	186	339	261
17	134	158	107	116	55	61	126	341	280
18	135	163	113	142	70	72	132	308	236

There are significant differences between place means and between variety means for sucrose (Z) and for total fermentable sugars (T). For gas due to the sugars originally present (G_T), only the place means differ significantly (Table III). When uniform amounts of alpha-

TABLE II

GAS PRODUCTION BY NATURAL AND ARTIFICIAL FLOURS

Sample number	Natural flours				Amylase-free flours with added beta- and alpha-amylases			
	First three hours	End of third to end of seventh hour	First five hours	Total gas	First three hours	End of third to end of seventh hour	First five hours	Total gas
	G_1	G_2	G_3	G_4	G_1'	G_2'	G_3'	G_4'
	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
1	233	108	293	617	308	333	520	879
2	216	291	395	1101	321	336	559	893
3	227	101	324	595	380	279	587	895
4	220	178	348	736	321	303	545	828
5	205	72	246	447	327	245	508	795
6	217	74	260	498	304	196	449	635
7	235	129	320	770	338	189	474	715
8	158	41	183	319	313	124	393	572
9	185	37	209	309	332	139	425	618
10	270	124	351	906	335	145	431	628
11	242	95	300	619	335	149	439	629
12	165	60	200	389	304	118	377	530
13	213	51	243	414	329	173	454	643
14	176	61	210	392	304	128	388	581
15	211	70	253	483	339	180	462	667
16	150	38	171	257	279	104	346	462
17	135	33	154	223	272	109	343	448
18	142	40	165	255	264	92	321	421

amylase were added to yeasted amylase-free doughs, there was a significant difference between the means for varieties and between means for places. Corresponding additions of beta-amylase gave significant differences between variety means only. The amount of gas formed corresponding to the gross diastatic activity for the natural flours ($G_4 - G_T = G_g$) shows a significant variation between means of varieties and places, as does the equivalent value G_g' for the artificial flours, although the significance is of a higher order in the latter case. The variance in the amount of gas G_n corresponding to the net diastatic activity in the natural flours is significant for differences between means of varieties only, but in the case of the artificial flours the variance of the corresponding value (G_n') is highly significant for differences between means of varieties and places.

The gas formed from the artificial flours shows a highly significant difference between means of places for each of the four time intervals, and between means of varieties for each time interval other than the first three hours. For the natural flours, however, in which at least one other variable is present, namely, the amount of alpha-amylase, the results are not so uniform. The variance between means of places

TABLE III

ANALYSIS OF VARIANCE FOR THE EFFECT OF WHEAT VARIETY AND LOCATION OF GROWTH ON SUCROSE, TOTAL SUGARS, GAS FORMED IN DIFFERENT TIME INTERVALS, AND ON GAS CORRESPONDING TO TOTAL SUGARS AND TO GROSS AND NET DIASTATIC ACTIVITY VALUES FOR NATURAL AND ARTIFICIAL FLOURS

Source of variation	Degrees of freedom	Z	T	G_T	G_g'	G_n'	G_β	G_g	G_n	G_α
Between means of varieties	5	2,391**	2,353*	534	42,288**	15,452**	6,769**	97,978*	69,471*	8,155**
Between means of places	2	9,554**	12,932**	9,083**	25,994**	16,136**	1,279	84,211*	67,794	1,939**
Remainder	10	410	518	291	1,896	998	498	19,846	17,898	223
		G_1	G_2	G_3	G_4	$G_1 + G_2$	G_1'	G_2'	G_3'	G_4'
Between means of varieties	5	1,697*	7,903*	9,078**	55,184	15,032*	530	16,951**	12,970**	51,545**
Between means of places	2	5,675**	6,000	16,756**	262,394**	21,062**	3,506**	7,829**	14,638**	53,726**
Remainder	10	501	1,767	891	18,427	2,182	326	841	664	1,855

* Significant.
** Highly significant.

is highly significant for the gassing periods up to the end of the fifth hour and for the total gas; and between means of varieties it is at least significant for periods up to the end of the seventh hour, but the variance between means of varieties for total gas and between means of places for the period from the fourth to the seventh hour is not significant.

Correlations for Sugars Originally Present and the Gas Produced from Them. The sugars originally present in the flours (T) correspond closely to the total gas produced from them (G_T), as shown by the correlation of 0.927 (Table IV). Their relationship to the gas produced by the natural flours during the different time intervals varies over a wide range, the correlation (0.810) being highest for the first three hours (G_1), but not even significant for the following four hours (G_2). If the gas produced by the sugars originally present (G_T) be compared with that produced in the different time intervals, the same condition holds, i.e., G_1 is the most and G_2 the least closely related.

For the artificial flours, the correlation between T or G_T and the gas formed in the different periods is similar to that for the natural flours, the actual values of the corresponding correlation coefficients being slightly higher for the artificial flours.

TABLE IV

CORRELATION COEFFICIENTS FOR TOTAL SUGARS AND GAS PRODUCTION

Natural flours			Artificial flours		
Variables correlated	T	G_T	Variables correlated	T	G_T'
G_T	0.927**	—	—	—	—
G_1	0.810**	0.774**	G_1'	0.780**	0.791**
G_2	0.356	0.334	G_2'	0.587*	0.524*
G_3	0.693**	0.675**	G_3'	0.740**	0.715**
G_4	0.542*	0.497*	G_4'	0.725**	0.655

Susceptible Starch. The sugars produced by beta-amylase activity (M_β), and used to measure the susceptible starch, are closely correlated with the corresponding gas production (G_β); $r = 0.947^{**}$. Sugars produced by alpha-amylase activity, also used to measure susceptible starch, are not quite so closely correlated with gas production (G_α); $r = 0.826^{**}$. The correlation between G_β and susceptible starch (S), as measured by the proportion of starch granules staining with iodine-congo red solution, though highly significant, is still lower; $r = 0.754^{**}$.

It has been shown by Dadswell and Gardner (1947) that there is a close relation between susceptible starch (M_β) and both the gross (A_g) and net (A_n) diastatic activities. Gas productions corresponding to

gross and net diastatic activities (i.e., G_g and G_n) were therefore correlated with M_β as a measure of susceptible starch and with the gas corresponding to it (G_β). The correlations are high for artificial

Variables	*Coefficient*	*Variables*	*Coefficient*
$G_g \times M_\beta$	0.577**	$G_g' \times M_\beta$	0.955**
$G_g \times M_\beta$	0.424	$G_g \times G_\beta$	0.927**
$G_n \times M_\beta$	0.428	$G_n' \times M_\beta$	0.911**
$G_n \times M_\beta$	0.464	$G_n' \times G_\beta$	0.864**

flours, but for the natural flours the only coefficient that is significant is that for $G_g \times M_\beta$.

M_β and G_β show a significant degree of correlation with the gas formed in the different periods in the natural flours and with that formed in all periods other than the first three hours in the case of the artificial flours (Table V). The values of the corresponding correlation coefficients are much higher for the artificial flours than for the natural flours in every period except the first three hours, when the reverse is the case. The closest relationship between susceptible starch (M_β or G_β) and gas production is in the fourth to seventh hours (G_2) for both the natural and artificial flours, and the least closely related was the first three hours of gas production (G_1).

TABLE V

CORRELATION COEFFICIENTS FOR GAS FORMED IN THE DIFFERENT PERIODS

Natural flours			Artificial flours		
Variables correlated	M_β	G_β	Variables correlated	M_β	G_β
G_1	0.519*	0.463*	G_1'	0.412	0.362
G_2	0.664**	0.726**	G_2'	0.948**	0.937**
G_3	0.676**	0.684**	G_3'	0.854**	0.836**
G_4	0.607**	0.632**	G_4'	0.918**	0.885**

Correlations were highest between amounts of sugar and gas pressures corresponding to the gross and net diastatic activity figures for the artificial flours, although the correlation coefficients for the natural flours were highly significant (Table VI). Both the net and gross diastatic activity figures were also closely correlated with the gas formed during all the different time intervals except the first three hours for the artificial and natural flours, the coefficients being higher for the former group. The gas pressures corresponding to the gross and net diastatic activity figures were in most cases more highly correlated with the gas formed during different time intervals than were the actual maltose values.

TABLE VI

CORRELATION COEFFICIENTS RELATING TO COMBINED ACTION OF STARCH AND AMYLASES

Natural flours					Artificial flours				
Variables correlated	A_g	G_g	A_n	G_n	Variables correlated	A_g'	G_g'	A_n'	G_n'
A_g	—	0.821**	—	—	A_g'	—	0.925**	—	—
A_n	—	—	—	0.835**	A_n'	—	—	—	0.925**
G_1	0.577**	0.716**	0.517*	0.699**	G_1'	0.495*	0.569*	0.514*	0.662**
G_2	0.886**	0.933**	0.925**	0.880**	G_2'	0.976**	0.961**	0.967**	0.925**
G_3	0.834**	0.920**	0.819**	0.875**	G_3'	0.929**	0.939**	0.937**	0.952**
G_4	0.821*	0.989**	0.865**	0.968**	G_4'	0.951**	0.976**	0.944**	0.979**

Alpha-amylase as measured by dextrin formation (Kent-Jones and Amos, 1940) was not significantly correlated with gas production during the first three hours, but as the fermentation proceeded the effect of this enzyme became more apparent. The dextrin figure was significantly correlated with the gas pressures corresponding to the gross and net diastatic activity figures, the coefficients being considerably higher than those for the corresponding relationship between the maltose values and dextrin.

Variables	*Coefficient*	*Variables*	*Coefficient*
$D \times G_1$	0.341	$D \times G_g$	0.822**
$D \times G_2$	0.788**	$D \times G_n$	0.836**
$D \times G_3$	0.622**		
$D \times G_4$	0.763**		

Estimation of Gas Production. Various combinations of the analytical data were used in an attempt to find the most satisfactory set for determining the gas formation of the natural flours in different time intervals. The best estimate of gas formed in the first three hours was achieved by using the factors percent of dextrin (D), total sugars originally present in the flour (T), and susceptible starch (S) as measured by the staining technique, the multiple correlation coefficient being 0.891, although the other combinations tried gave values which were almost as satisfactory. The best estimates for the two periods, the first five hours (G_3) and from the end of the third to the end of the seventh hour (G_2), were achieved by using the net diastatic activity figure (A_n) and the sugars originally present in the flours (T), the values for the over-all correlations being 0.965 and 0.935 respectively. Susceptible starch (M_β), the logarithm of the dextrin figure (log D), and the sugars originally present (T) gave the highest multiple correlation coefficient (0.980) for the total gas formation.

For the artificial flours the least satisfactory estimates were those for the first three hours (G_1'), the best over-all correlation obtained

in this case (0.793) being that relating the net diastatic activity (A_n'), the sugars originally present (T), and G_1'. For the other time intervals the use of the sugars originally present (T) combined with either the gross (A_g') or net (A_n') diastatic activity gave multiple correlation coefficients of the same order as those obtained for the natural flours using the same variants.

TABLE VII

STATISTICS RELATING TO PREDICTION OF GAS PRODUCTION

Natural flours					Artificial flours				
Independent variables	Dependent variable				Independent variables	Dependent variable			
	G_1	G_2	G_3	G_4		G_1'	G_2'	G_3'	G_4'
CORRELATION COEFFICIENTS									
$M\beta$, D, T	0.873**	0.915**	0.941**	0.938**					
$M\beta$, T after adjustment for D	0.856**	0.755**	0.903**	0.844**	$M\beta$, T	0.780**	0.932**	0.953**	0.967**
A_n, T	0.875**	0.935**	0.965**	0.931**	A_n', T	0.793**	0.942**	0.983**	0.955**
A_g, T	0.852**	0.886**	0.917**	0.850**	A_g', T	0.788**	0.980**	0.975**	0.987**
$G\beta$, G_n, G_T	0.904**	0.951**	0.994**	0.999**	$G\beta$, G_n', G_T	0.866**	0.976**	0.988**	0.999**
STANDARD ERROR OF ESTIMATE									
$M\beta$, D, T	20.5	28.4	26.7	92.0	$M\beta$, T	18.4	30.9	24.9	41.0
A_n, T	19.7	24.1	20.0	93.7	A_n', T	17.9	28.5	15.2	47.3
A_g, T	21.3	31.5	30.6	135.1	A_g', T	18.1	16.9	18.1	25.6
$G\beta$, G_n, G_T	18.0	21.7	8.6	0	$G\beta'$, G_n', G_T	15.2	19.3	13.1	0
PER CENT OF VARIANCE NOT ACCOUNTED FOR BY REGRESSION									
$M\beta$, D, T	28.8	19.8	13.8	14.6					
$M\beta$, T after adjustment for D	30.5	49.2	21.2	32.8	$M\beta$, T	44.4	14.9	10.5	7.5
A_n, T	26.6	14.3	7.7	15.2	A_n', T	42.1	12.7	3.9	9.9
A_g, T	31.1	24.4	18.1	31.5	A_g', T	43.0	4.5	5.5	2.9
$G\beta$, G_n, G_T	22.2	11.6	1.4	0	$G\beta$, G_n', G_T	30.3	5.8	2.9	0

A measure of the differences between actual and estimated gas production in the various time intervals is given by the standard error of estimate (Table VII). The estimation of gas pressure developed during the first three hours is poor, regardless of the variables used for prediction. The most satisfactory predictions of G_2 and G_3 are naturally based on gas pressure values (G_β, G_n, G_T), while the best estimates for these two time intervals based on sugar values depend on the net diastatic activity figure (A_n) and the sugar originally present (T). The gross diastatic activity figure is in each case less satisfactory than the net diastatic activity.

The error of estimate for the artificial flours, expressed as percentage of variance not accounted for, is only slightly less than for the

TABLE VIII

STATISTICS RELATING TO PREDICTION OF GAS PRODUCTION CORRESPONDING TO GROSS AND NET DIASTATIC ACTIVITY

Statistics	Natural flours			Artificial flours		
	Independent variables	Dep. variable		Independent variables	Dep. variable	
		G_g	G_n		G_g'	G_n'
Corr. coeff.	M_β, D	0.891**	0.857**			
Corr. coeff. after adjusting for D	M_β	0.607**	0.338	M_β	0.955**	0.911**
Error of est.	M_β, D	108.3	108.3	M_β	39.3	35.7
Variance not accounted for, %	M_β, D	23.3	30.1			
Ditto, after adjusting for D	M_β	67.4	94.5	M_β	9.3	18.1

natural flours. However, such an observation is open to question since in one series the amylase level is variable and in the other it is constant. In order that a theoretically more satisfactory comparison can be made between the two series of flours, it can be carried out after allowance has been made for the variance due to alpha-amylase (D). This correction has been made experimentally for the artificial series and mathematically for the natural series of flours. With the exception of the first three hours, the proportion of the total variance unaccounted for in the natural flours greatly exceeds that unaccounted for in the artificial series or, expressing it differently, the importance of susceptible starch in determining the magnitude of the standard error is much greater in the case of the artificial flours. In the case of the natural flours much more of the original variance has evidently to be accounted for in some other way, such as by an unknown variable.

When the variants considered are M_β, D, and T, the actual standard error, expressed as mm. of pressure per 10 g. of flour, is approximately the same for the natural and artificial flours for all intervals except the total gas (G_4, G_4'). However, for the variants T and A_g or A_n, the standard error of the natural flours is greater than that for the artificial flours in all periods except one.

The magnitude of the residual variance which is unaccounted for in either flour series could not be explained by what is known of the standard deviation of the variants concerned. Thus there may also be an unknown variant in the starch substrate that survives the treatment given to destroy the amylases and is common to both the natural and artificial flour series. If there were a difference in susceptibility of the undamaged starch to a raw starch-splitting amylase, it would

be expected to be a contributing factor in this portion of the standard error.

When the standard error of estimate of the gas pressures corresponding to the gross and net diastatic activity figures of the natural flours is compared with that for the artificial series (Table VIII), it is evident that these exhibit the same peculiarities as does the total gas (G_4, G_4'), i.e., a better proportional correction of the error of estimate is effected by these variables for the artificial flours and the final error is numerically lower than for the natural flours. The standard error of estimate of the net and gross diastatic activity figures for the same series of flours (Dadswell and Gardner, 1947) showed a different trend,

TABLE IX

ESTIMATION OF GAS FORMED IN DIFFERENT PERIODS IN NATURAL FLOURS

G_1

$0.2094\ A_g + 0.5249\ T + 51.96$
$0.02923\ M_\beta + 6.208\ D + 0.6039\ T + 23.27$
$0.4395\ A_n + 0.5543\ T + 47.22$
$0.05342\ G_\beta + 0.08998\ G_n + 0.6081\ G_T + 74.48$

G_2

$1.072\ A_g - 0.01989\ T - 44.64$
$0.8381\ M_\beta + 21.61\ D + 0.1872\ T - 137.70$
$1.918\ A_n + 0.1784\ T - 60.95$
$0.5305\ G_\beta + 0.2263\ G_n - 0.04822\ G_T - 20.06$

G_3

$0.8924\ A_g + 0.6003\ T + 3.394$
$0.5465\ M_\beta + 19.84\ D + 0.8163\ T - 83.73$
$1.677\ A_n + 0.7538\ T - 12.12$
$0.4155\ G_\beta + 0.2235\ G_n + 0.7297\ G_T + 37.15$

G_4

$3.263\ A_g + 1.161\ T - 170.5$
$1.400\ M_\beta + 85.80\ D + 2.120\ T - 560.3$
$6.333\ A_n + 1.694\ T - 231.93$

the percentage of reduction effected by consideration of susceptible starch being the same for the gross diastatic activity in the two groups of flours, but greater in the case of the artificial flours for the net diastatic activity. The actual reduction is much greater for the latter series, the final values being such that the natural flours give much lower errors of estimate than those of the artificial series.

The combination A_g, T, or A_n, T each contain one variant involving the interaction between susceptible starch, whether in the form of damaged starch or of undamaged starch of varying susceptibility, and any raw starch-splitting amylases. Under conditions of varying alpha-amylase content the gross diastatic activity (A_g), although intimately related to susceptible starch and the net diastatic activity, is less satisfactory when judged on the basis of variance unaccounted for

than are those two factors when considered as separate entities in the estimation of gas formation. Under conditions of uniform kind and concentration of amylase, however, the gross diastatic activity gives a slightly better estimate of potential gas formation in the later periods than does the net diastatic activity figure, especially between the end of the third hour and the end of the seventh hour.

The equations relating the potential gas production in each time interval to the different variables used are shown in Table IX for the natural flours and in Table X for the artificial flours. These equations were obtained as customary by use of regression coefficients.

TABLE X

ESTIMATION OF GAS FORMED IN DIFFERENT PERIODS IN ARTIFICIAL FLOURS

G_1'
$0.02292\ M_\beta + 0.4219\ T + 218.0$
$0.02883\ A_g' + 0.3946\ T + 214.8$
$0.04774\ A_n' + 0.3864\ T + 212.9$
$-0.2736\ G_\beta + 0.2683\ G_n' + 0.3605\ G_T + 189.66$
G_2'
$2.3645\ M_\beta + 0.2332\ T - 38.88$
$0.6511\ A_g' + 0.1660\ T - 103.7$
$0.8511\ A_n' + 0.1826\ T - 123.15$
$1.100\ G_\beta + 0.2224\ G_n' + 0.4108\ G_T - 94.46$
G_3'
$1.6802\ M_\beta + 0.6348\ T + 177.88$
$0.5078\ A_g' + 0.5335\ T + 126.8$
$0.6840\ A_n' + 0.5298\ T + 108.3$
$0.4643\ G_\beta + 0.4616\ G_n' + 0.7171\ G_T + 100.7$
G_4'
$3.762\ M_\beta + 1.050\ T + 143.86$
$1.0447\ A_g' + 0.9328\ T + 39.80$
$1.369\ A_n' + 0.9559\ T + 8.252$

A comparison of the estimated and actual gas production has been made graphically in Figures 1 and 2. The natural flour samples have been arranged in order of increasing gas production. The gas actually produced by the artificial flours and the calculated values for both the natural and artificial flours have been arranged in the same order. It is at once obvious that the agreement between the actual and estimated gas production figures for the artificial flours is much better than it is for the natural flours. It is also evident that the estimated gas production of the natural flours is highly correlated with the actual and estimated gas production of the artificial flours.

The marked disagreement between the actual and estimated values in some samples may be due to one or more variants which must still be accounted for. A better agreement is observed when the estimation is based on the gas pressures G_β, G_n, and G_T. M_β and T are

closely correlated with G_β and G_T. If a better correlation could be obtained between A_n and G_n, as in the case of A_n' and G_n', a better estimate of potential gas production would be possible.

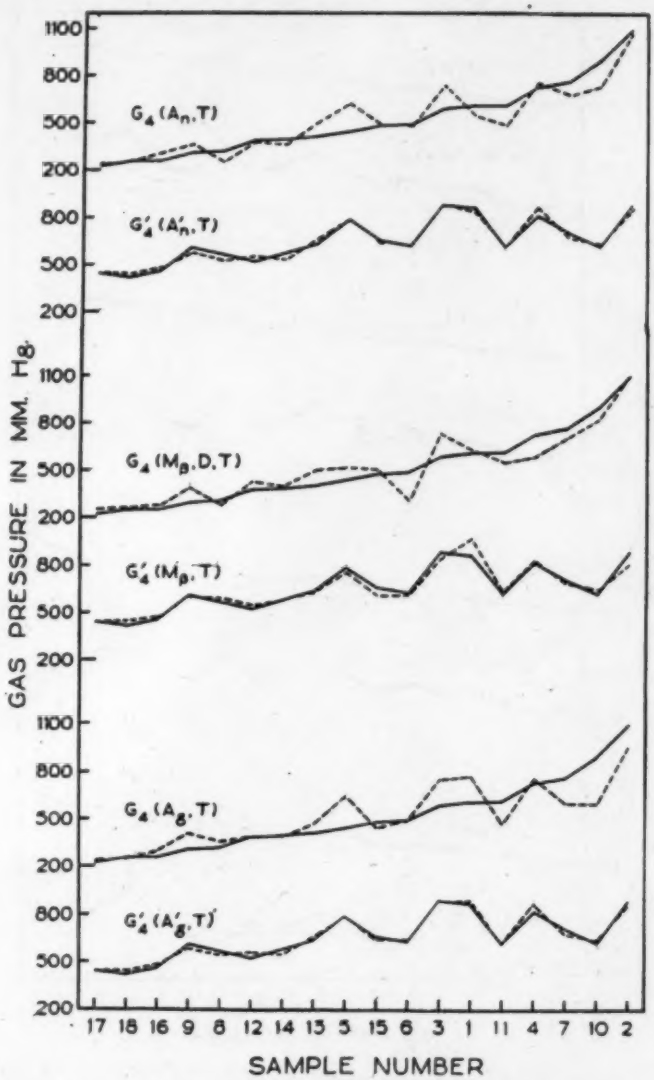

Fig. 1. Actual (———) and estimated (------) values of the total gas produced in natural flours (G_4) and in artificial flours (G_4'), based on equations in Tables IX and X.

Figure 3 was prepared from the information available regarding the effect of variations in susceptible starch, alpha-amylase, and sugars originally present on the gas produced in relation to time. The maximum and minimum values used for each variant are those for the

range covered by the samples studied in this experiment. Each variant has been treated separately and in various combinations with the others in order to show the effect on gas production of this range from

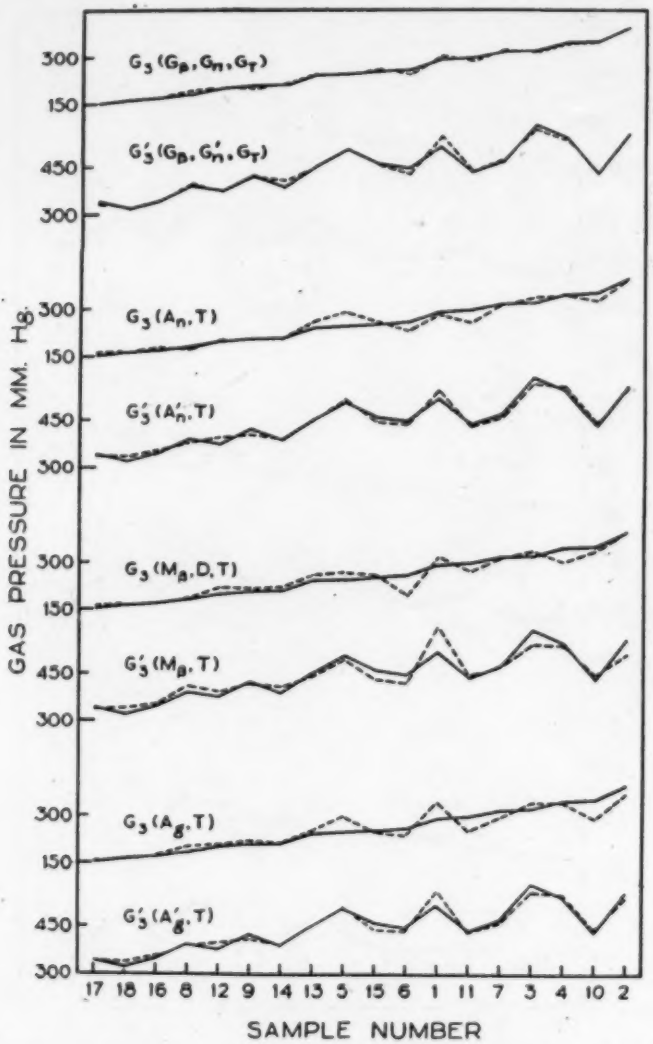

Fig. 2. Actual (———) and estimated (- - - - - -) values of the gas produced during the first five hours in natural flours (G_3) and in artificial flours (G_3'), based on equations in Tables IX and X.

minimum to maximum values. Variation in M_β has little effect on gas production in the first three hours but has its greatest effect in the fourth hour, after which it gradually declines in importance. D has some effect during the first three hours, is at a maximum in the fourth hour, and maintains a substantial but slowly declining gas production

subsequently. T exerts its maximum influence in the first three hours, after which its effects diminish rapidly. Maximum values of T and D together give the highest as well as the most sustained gas production, while maximum values in M_β and T result in a less prolonged gas production.

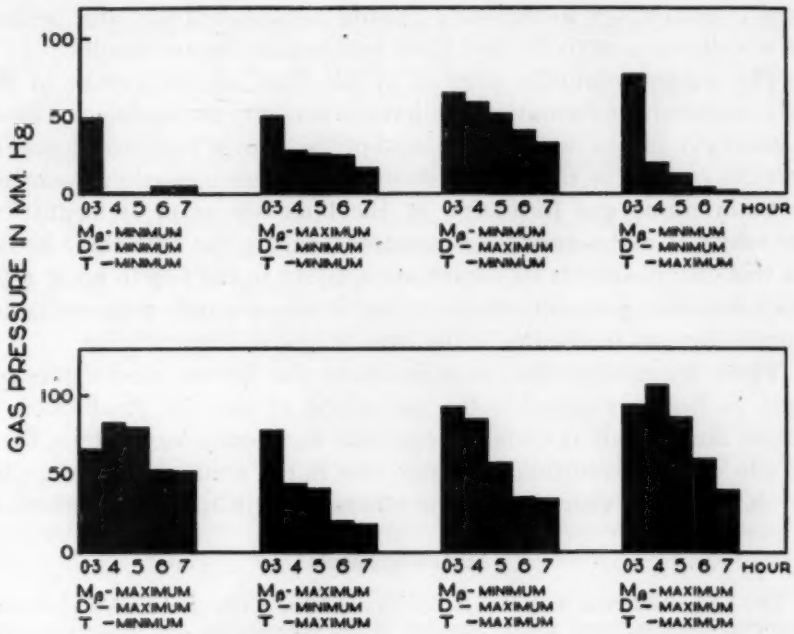

Fig. 3. Effect of variation in susceptible starch (M_β), alpha-amylase (D), and sugars originally present (T) on hourly gas production, as based on the equations in Table IX. (The values designated 0-3 hour represent the average gas production per hour during the first three hours.)

Summary

Flours prepared from six varieties of wheat, each grown at three places, have been studied with reference to their gas production.

Variety and place of growth were found to be related to the sugars originally present in these flours and to the gas formed in doughs made from an artificial series of flours having a constant amylase content. The gas formed by the natural flours varies in its relation to variety and place depending on the interval of gas production considered.

There is a high correlation between both sugars originally present and those formed as a result of amylase action and the gas formed from them under conditions of constant or variable amylase concentration.

The gas produced in different time intervals is significantly correlated with the gross and net diastatic activity figures in the case of both the natural and artificial flours.

Estimations of potential gas formation in different gassing periods under conditions of variable alpha-amylase content were more satisfactory when based on the net diastatic activity figure and the sugars originally present than when based on the latter factor and the gross diastatic activity. When constant amounts of alpha- and beta-amylase were present as in artificial flours, the two methods were equally satisfactory in the early gassing periods, but for later periods the net diastatic activity and total sugars gave better results.

The sugars originally present in the flour are important in the early stages of gas formation but have practically no significance later. A variation in the amount of susceptible starch becomes apparent after the end of the third hour, showing that this is partly responsible for maintaining gas formation in the later stages of fermentation. The effect of alpha-amylase is apparent during the first three hours, but this enzyme exerts its maximum activity in the fourth hour, after which its effect gradually declines; but it also is partly responsible for maintaining gas formation in the later stages of fermentation.

There is evidence that, in addition to the factors generally recognized as being responsible for variations in the gas production of natural flours, such as variable sugar and alpha-amylase content, there are one or more additional factors; one is the amount of susceptible starch, while the character of the others has still to be determined.

Acknowledgments

Dr. J. A. Anderson and Mr. A. E. Paull of the Grain Research Laboratory, Winnipeg, Canada, have greatly assisted in the organization and statistical interpretation of the material presented, for which the authors are very grateful.

The authors also wish to thank Mr. W. R. Jewell, Agricultural Research Chemist, Department of Agriculture of Victoria, for providing the flour samples used in this work. W. S. Kimpton & Sons, Flour Millers, generously provided the wheat used for the enzyme preparations. The encouragement and interest shown by the late Professor W. J. Young and by Professor V. M. Trikojus of the Department of Biochemistry of the University of Melbourne have been very much appreciated.

The University of Melbourne provided the Research Scholarships held by the authors during the time that the work was carried out.

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DOUGH OXIDATION AND MIXING STUDIES. VII. THE ROLE OF OXYGEN IN DOUGH MIXING

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(Presented at the Annual Meeting, May 1947; received for publication June 30, 1947)

The research reported here is concerned with the effects of molecular or gaseous oxygen in dough mixing. In a previous investigation on this subject, Baker and Mize (1937) obtained improved bread quality upon mixing doughs in oxygen for normal intervals; but their work tended to emphasize the harmful effects of overmixing in oxygen on dough and bread quality. Later, Freilich and Frey (1939, 1941) reported the following effects when doughs were mixed in oxygen in the Hobart-Swanson mixer for normal time intervals: (a) the protease activity of papain was inhibited in the patent flour to which it was added, (b) the detrimental effects of reducing matter added to patent flour were minimized, and (c) the quality of bread made from low grade flour was improved. Differences in the condition of the doughs after fermentation and in loaf volume and bread quality were the criteria used for noting these effects. The present research evaluates the effects of oxygen in dough mixing in terms of dough development and bread quality.

Materials and Methods

Patent or bakers grade flours were used, and dough development was followed by means of farinograph curves. Doughs were mixed and bread baked both by the straight and sponge procedures, employing the following formula:

Flour—100% (300 g), water (variable)—62–64%, yeast—2%, sucrose—5%, salt—2%, shortening—3%.

In the experiments with papain, Figures 6 and 7, 3% milk powder was used in addition to the other ingredients.

The Brabender Farinograph was used in most of the mixing experiments. It was fitted with a cover having a rubber gasket which also fitted the rim of the mixing bowl. The cover was provided with two openings, one for admitting the gas used and the other for connection with a manometer. Mixing was done under slight positive pressure (about 10 mm of Hg).

The straight doughs were mixed in the farinograph (unless otherwise noted) for sufficient time to determine whether a peak occurred in the farinograph curve as dough development proceeded. This usually occurred between 12 and 18 minutes. After about two hours'

fermentation at 30°C, when a dough volume of 1180 ml was attained, 480-g portions were rounded, molded by machine, proofed at 40°C to the top of the pan, and baked for 30 minutes at 215°C. In some experiments, the dough was remixed after fermentation and this is so indicated in each instance.

The sponges (60% flour, 37% water, and the yeast)[1] were mixed in the Hobart-Swanson mixer for two minutes, fermented for three and one-half hours at 30°C, and then mixed with the dough ingredients in the farinograph long enough (approximately 15 minutes) to show whether or not a peak in dough development was being indicated. After a 20-minute dough time, 480-g portions of the doughs were rounded, molded, proofed, and baked in the same manner as were the straight doughs.

Effects of Oxygen on Dough Development

Good development is a condition attained by doughs during mixing, and is characterized by maximum dough consistency or firmness and good elasticity. These properties are correlated with optimum bread quality factors, such as loaf volume, texture, and grain. The farinograph curve gives a good index of the course of dough development during mixing, and mixing time, consistency, and elasticity of the dough may be evaluated from the curve.

The effects of mixing straight doughs in oxygen and in nitrogen are shown in Figure 1. Oxygen had a very profound effect on the dough. The curve for a straight dough mixed in oxygen before fermentation shows good consistency and elasticity and goes through a definite peak. But the curve for a straight dough mixed in nitrogen before fermentation is flat, showing practically no development or increase in firmness during mixing. These curves indicate the fundamental importance of oxygen as a factor in proper dough development; such development is evidently unobtainable in the absence of oxygen.

It is also evident that oxygen is most effective when incorporated into the dough during the original mixing. The dough which was mixed in nitrogen, then remixed in oxygen after fermentation, does show development, but it required 30 minutes to attain that stage, as against 12 minutes for the dough mixed in oxygen originally. The dough which was remixed in nitrogen after fermentation, following an original mixing in nitrogen, showed little development even after 30 minutes of mixing, again indicating the essential need of oxygen in dough development.

That satisfactory dough development can be achieved by incorporation of oxygen after fermentation is illustrated in Figure 2. All

[1] The amounts used in the sponge were—180 g flour, 110 ml water, 6 g yeast (300 g = 100%).

of the doughs from which these loaves were baked were mixed in nitrogen for four minutes in the Hobart-Swanson mixer (this mixer develops doughs much more rapidly than the farinograph). After a normal fermentation period of about two hours, until a dough volume

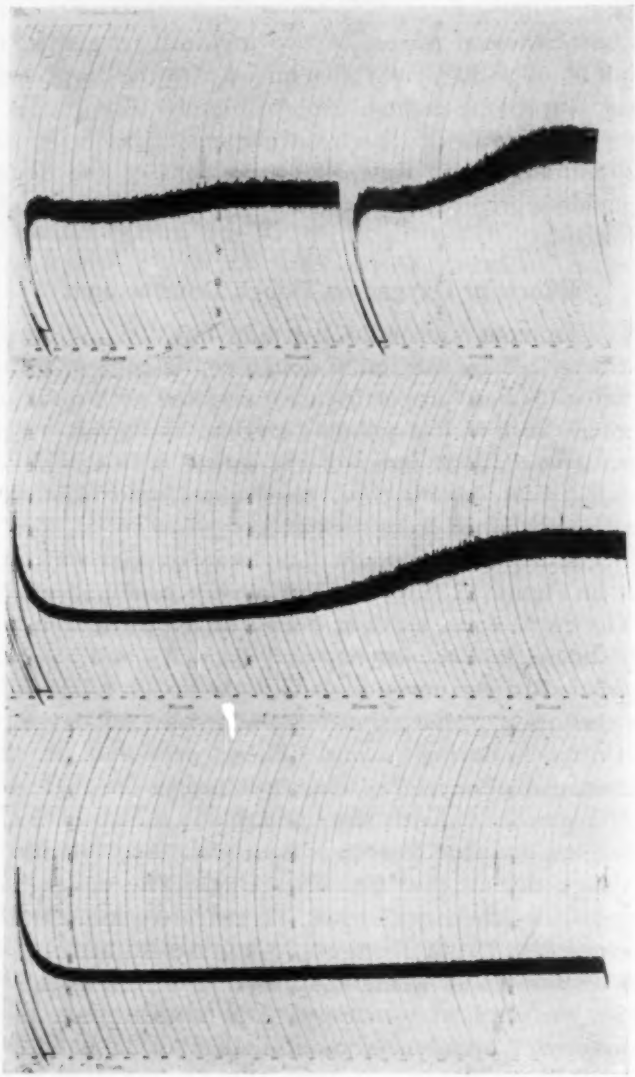

Fig. 1. Farinograph curves of straight doughs mixed in nitrogen and oxygen.
Top, left—Dough mixed in nitrogen before fermentation.
Top, right—Dough mixed in oxygen before fermentation.
Center—Dough mixed in oxygen after fermentation, following original mixing in nitrogen.
Bottom—Dough mixed in nitrogen after fermentation, following original mixing in nitrogen.

of 1180 ml was reached, doughs No. 1, 2, and 3 were remixed in the same mixer in air for one, five, and ten minutes respectively, and doughs No. 4, 5, and 6 were remixed in the same mixer in nitrogen, also for one, five, and ten minutes. Loaves 2 and 3 were of normal volume and texture, indicating that a 5–10 minute remix in air provided sufficient oxygen for proper dough development. But all the loaves baked from doughs mixed in nitrogen showed inferior volume and texture due to lack of dough development during remixing in the absence of oxygen.

Fig. 2. Bread baked from straight doughs which were mixed in nitrogen, fermented, and remixed in air or nitrogen.
Dough No. 1—Remixed in air for 1 minute.
Dough No. 2—Remixed in air for 5 minutes.
Dough No. 3—Remixed in air for 10 minutes.
Dough No. 4—Remixed in nitrogen for 1 minute.
Dough No. 5—Remixed in nitrogen for 5 minutes.
Dough No. 6—Remixed in nitrogen for 10 minutes.

Figure 3 illustrates the effects of mixing fermented sponges with the dough ingredients in air, nitrogen, and oxygen. The oxygen curve shows much higher consistency[2] and shorter mixing time than the nitrogen curve; the air curve shows similar effects of lesser magnitude. Loaf quality showed the same order of improvement, from mixing in nitrogen to mixing in oxygen. Thus, it is concluded that oxygen is essential in the mixing of sponge doughs, as well as in the mixing of straight doughs, if satisfactory dough development and loaf quality are to be obtained.

Effects of Oxygen in Doughs Made with Different Bread Flours

The farinograph curves in Figures 4 and 5 illustrate the progressive effects of increasing concentrations of oxygen in the mixing of doughs made from three samples of Northwestern flour and three samples of Kansas flour. These were all patent flours, 72% extraction. They were used in straight doughs which were mixed in nitrogen, air, and oxygen.

[2] "Consistency" is the term which appears on the graph paper used in the farinograph, to designate values on the vertical axis from 0 to 1000; "higher consistency" refers to these numerical values.

The curves for doughs mixed in nitrogen show little change from beginning to end of mixing, indicating a lack of dough development. Here the mixing times are difficult to estimate because the curves do not go through a definite peak.

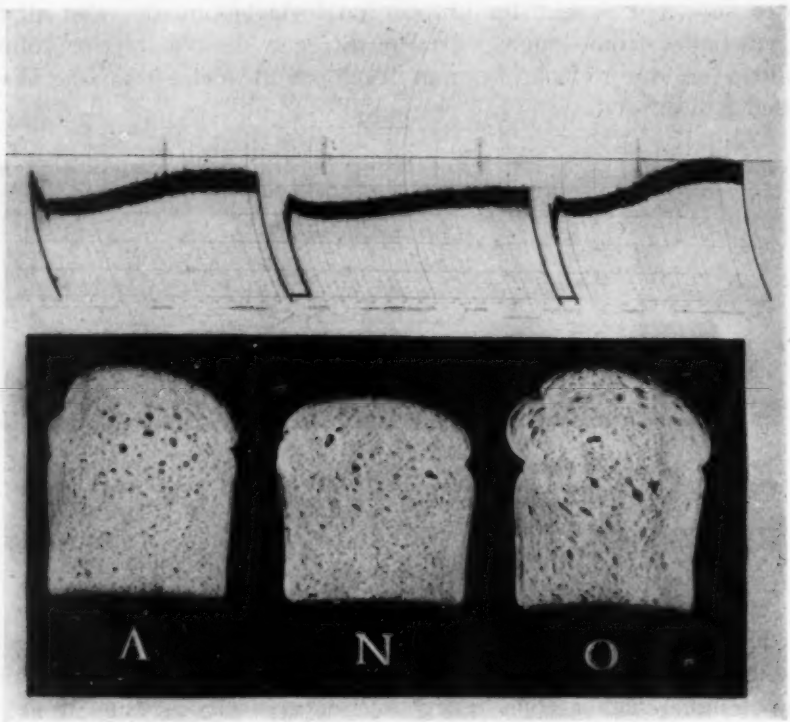

Fig. 3. Farinograph curves and bread baked from sponge doughs. Sponges all mixed in air before fermentation.
Fermented sponges mixed with dough ingredients in:
A = air
N = nitrogen
O = oxygen

The curves for the doughs mixed in air and in oxygen show progressively better dough development, as evidenced by higher consistency and greater elasticity (indicated by increased width at the peaks of the curves). Oxygen has a tendency to shorten the mixing time required for maximum dough development.

Mixing in nitrogen always resulted in bread of inferior volume and quality (Figures 4 and 5). The bread produced from the doughs mixed in air was much superior to the corresponding nitrogen loaves in all respects. Mixing in oxygen produced additional improvement in volume and quality, though not to the extent indicated by air over

nitrogen. Two of the six flours showed no improvement due to oxygen as compared to air. It was therefore indicated that, though the oxygen requirements of different flours may vary in degree, the total lack of oxygen in mixing prevents satisfactory dough development, with a consequent inferiority in the quality of the resulting bread.

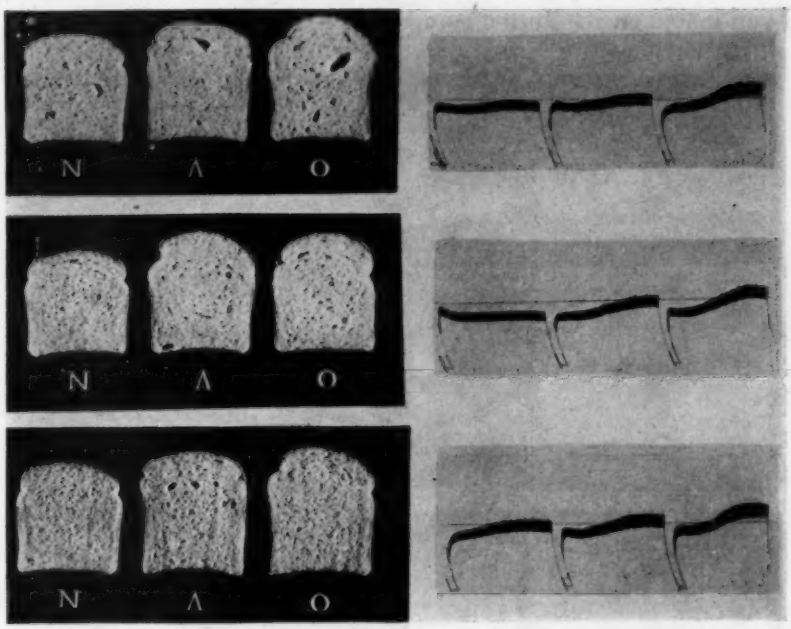

Fig. 4. Farinograph curves and bread baked from straight doughs made from different flours. Top row—Northwestern special patent; middle row—Northwestern bakers flour; bottom row—Northwestern patent flour. Before fermentation, doughs were mixed in: N = nitrogen, A = air, O = oxygen. See Table I for additional data.

The farinograph and baking data for these flours are given in Table I. From the dough times, it is seen that oxygen retarded fermentation in its initial stages, but that the rate of gas production was back to normal or somewhat faster during the proofing stage. This initial effect of oxygen may be explained on the basis of aerobic respiration. Yeast metabolism may be either anaerobic (fermentation) or aerobic (respiration). The presence of oxygen favors respiration, so that fermentation is retarded in the dough until the oxygen is used up. This effect in sugar solutions has long been known. For a discussion of the effect, see Werkman (1946).

Effects of Oxygen in Doughs Containing Added Protease

The beneficial effects of oxygen on dough development have been shown to be immediate, that is, they become apparent during the first

few minutes of dough mixing. The harmful effects of protease activity, on the other hand, are known to be delayed, particularly when protease is present at low concentration. In patent or bakers grade flours, the protease content is so minute as to be of little practical significance. It would therefore seem that the effects of oxygen during dough mixing do not involve protease activity, but that the oxygen probably acts on the gluten complex.

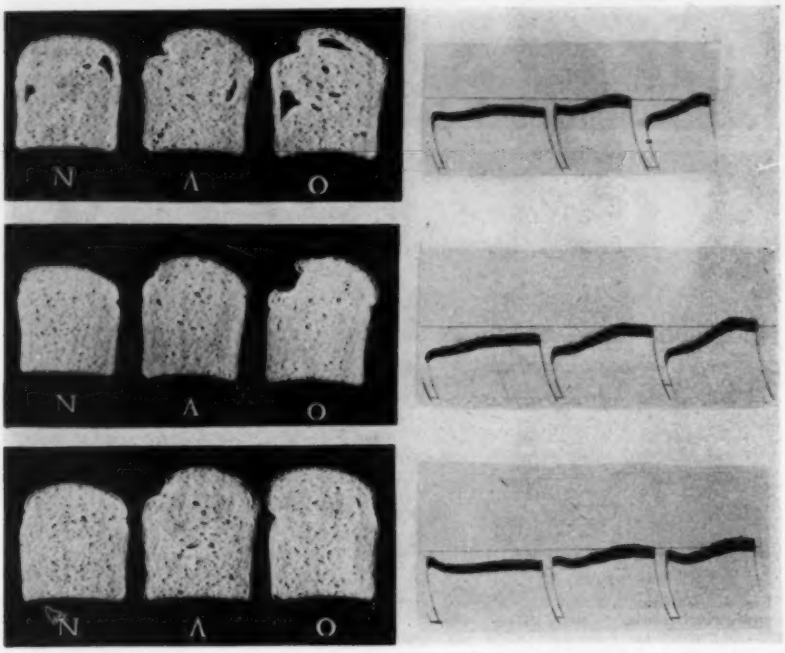

Fig. 5. Farinograph curves and bread baked from straight doughs made from different flours. Top row—Kansas hard winter patent; middle row—Kansas flour; bottom row—Kansas bakers flour. Before fermentation, doughs were mixed in: N = nitrogen, A = air, O = oxygen. See Table I for additional data.

To substantiate the theory that the oxygen effect during mixing is independent of protease activity of the papain type, which manifests itself only during subsequent processing of the dough, straight doughs with and without added papain were mixed in nitrogen and in oxygen in the farinograph. The curves showed the usual effects of mixing in oxygen, that is, increased consistency and elasticity, and shorter mixing time, but no effects of comparable magnitude were indicated due to the presence of papain during mixing (Figure 6). After several hours of fermentation, however, the effects of papain became apparent, as evidenced by inferior bread quality in loaves 2 and 3, made from doughs mixed in nitrogen. Papain produced no harmful effects in the doughs mixed in oxygen (loaves 5 and 6).

TABLE I

FARINOGRAPH AND BAKING DATA FOR DIFFERENT FLOURS
(Straight doughs, mixed in nitrogen, air, and oxygen)

Type of flour	Gas used in mixing	Mixing time	Consistency	Dough time	Pan proof time	Loaf volume	Bread quality
		Min.	*B.U.*[2]	*Min.*	*Min.*	*ml*	
Northwestern special patent	Nitrogen	12.0[1]	450	113	52	1700	Dense; below normal.
Northwestern special patent	Air	13.0	500	113	52	1880	Close to normal; slightly dense.
Northwestern special patent	Oxygen	13.0	570	131	51	1920	Normal, though slightly open.
Northwestern bakers flour	Nitrogen	13.0[1]	390	112	56	1590	Dense; slightly coarse, below normal.
Northwestern bakers flour	Air	13.5	490	110	56	1830	Normal.
Northwestern bakers flour	Oxygen	12.5	550	124	55	1830	Normal; slightly over-oxidized.
Northwestern patent	Nitrogen	12.5[1]	490	105	50	1640	Dense; below normal.
Northwestern patent	Air	13.0	520	108	50	1830	Close to normal.
Northwestern patent	Oxygen	12.0	590	122	52	1910	Normal.
Kansas hard winter patent	Nitrogen	9.5[1]	420	105	53	1680	Slightly dense; below normal.
Kansas hard winter patent	Air	8.5	490	106	59	1880	Close to normal.
Kansas hard winter patent	Oxygen	8.75	510	119	56	1880	Close to normal.
Kansas bakers flour	Nitrogen	12.5[1]	400	111	55	1660	Dense; below normal.
Kansas bakers flour	Air	11.0	480	114	54	1940	Normal.
Kansas bakers flour	Oxygen	10.5	520	126	52	1910	Slightly finer grain than in air-mixed loaf.
Kansas flour	Nitrogen	13.0[1]	420	106	56	1740	Dense; below normal.
Kansas flour	Air	10.25	480	110	56	1910	Normal.
Kansas flour	Oxygen	9.75	520	126	56	1970	Normal; rough break.

[1] Mixing time indefinite in nitrogen.
[2] B. U. = Brabender Units.

In another experiment, doughs with and without added papain were mixed in the farinograph in nitrogen and in oxygen, then remixed in the farinograph in air, after several hours of fermentation. Following the remixing, the doughs were allowed to rest for 20 minutes, and then were rounded, molded, proofed, and baked as usual. The farinograph curves in Figure 7 showed significant differences due to the action of papain, in contrast to the curves in Figure 6. The papain produced more rapid dough development during remixing, as indi-

cated by higher consistency in the doughs originally mixed in nitrogen, and shorter mixing times in all doughs. For the doughs originally mixed in nitrogen, bread quality was related to dough development; the papain doughs showed better development during remixing and produced better bread than the control. The papain doughs (No. 5 and 6) originally mixed in oxygen produced satisfactory bread, comparable to the control bread (No. 4), and better than bread from doughs mixed in nitrogen (No. 1–3).

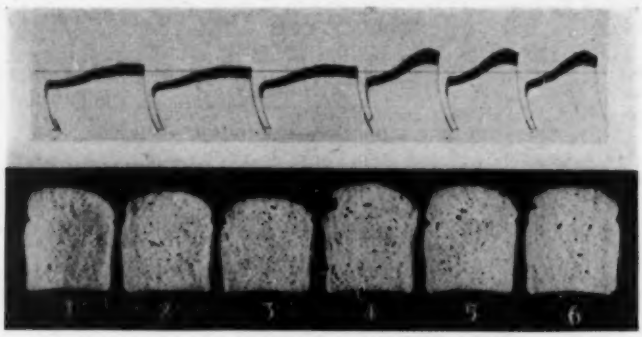

Fig. 6. Effect of added protease (papain) upon farinograph curves and bread baked from straight doughs mixed in nitrogen or oxygen.

Doughs mixed in nitrogen before fermentation:
Dough No. 1—No added papain.
Dough No. 2—5 mg papain.
Dough No. 3—10 mg papain.

Doughs mixed in oxygen before fermentation:
Dough No. 4—No added papain.
Dough No. 5—5 mg papain.
Dough No. 6—10 mg papain.

This phase of the investigation of the effects of oxygen in mixing doughs of high and low protease content may be summarized as follows:

1. In patent or bakers grade flours, in which the protease content is known to be practically insignificant, the beneficial effects of oxygen upon dough development during mixing, as shown in farinograph curves, are independent of any protease that may be present.

2. In doughs made from flours containing added protease, the effects of oxygen during dough mixing are also independent of protease activity, as shown by the farinograph curves. However, the harmful effects of protease in these doughs become apparent during subsequent processing after fermentation, as evidenced by inferior bread quality. These harmful effects of the protease are minimized and bread quality is normal when oxygen is present in sufficient quantities during mixing or during subsequent processing.

These results, therefore, indicate that the effects of oxygen during dough mixing are independent of any protease of the papain type that may be present in the flour. Oxygen produced effects that were immediately apparent, whereas the effects of added papain, both in the dough and in the resulting bread, became evident only after several hours of dough fermentation.

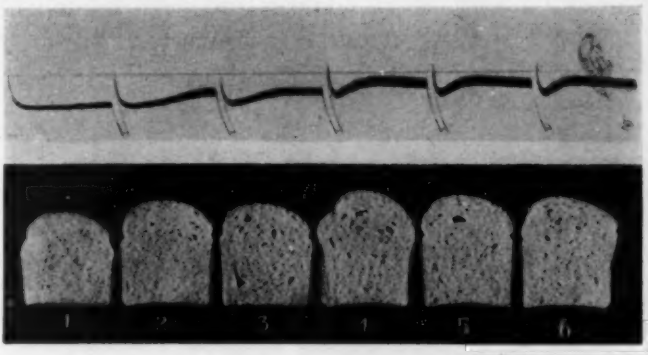

Fig. 7. Effect of added protease (papain) upon farinograph curves and bread baked from straight doughs mixed in nitrogen or oxygen, fermented and then remixed in air.

Doughs mixed in nitrogen before fermentation and remixed in air:
Dough No. 1—No added papain.
Dough No. 2—5 mg papain.
Dough No. 3—10 mg papain.

Doughs mixed in oxygen before fermentation and remixed in air:
Dough No. 4—No added papain.
Dough No. 5—5 mg papain.
Dough No. 6—10 mg papain.

Mechanism Involved in the Effects of Oxygen

The effects of oxygen in dough mixing were so pronounced that fundamental changes, possibly chemical in nature, were indicated. Since these changes were effected by molecular oxygen it appeared probable that an enzymic oxidation mechanism was involved. If this were so, it should be possible either to inhibit the reaction by inhibiting or inactivating the enzymes, or to obtain more pronounced effects by using a higher concentration of oxidizing enzyme. Both of these possibilities were investigated.

Figure 8 shows the effects of 0.5% cuprous chloride, an enzyme inhibitor, on a straight dough mixed in oxygen in the farinograph. The curve is very much like that obtained with a straight dough from the same flour, but mixed in nitrogen and containing no added cuprous chloride (Figure 1), indicating the inhibition of the mechanism involved in the oxygen effect. The enzyme activity of the yeast in the dough was also inhibited by the cuprous chloride.

Indirect evidence tending to confirm the presence of an oxidizing enzyme system was obtained by adding small amounts of quinua flour to the dough. *Quinua polylepis*, of the rose family, is a native-grown Bolivian grain widely used as food by the Indians;[3] it is not a wheat variety. Quinua flour appears to have a much higher concentration of oxidizing enzyme than wheat. Ten percent of the regular flour was replaced by an equal amount of the quinua flour in doughs which were mixed in air and in nitrogen, as compared to a control dough mixed in air. In another test, 10% quinua flour was heated in water to 95°C[4]

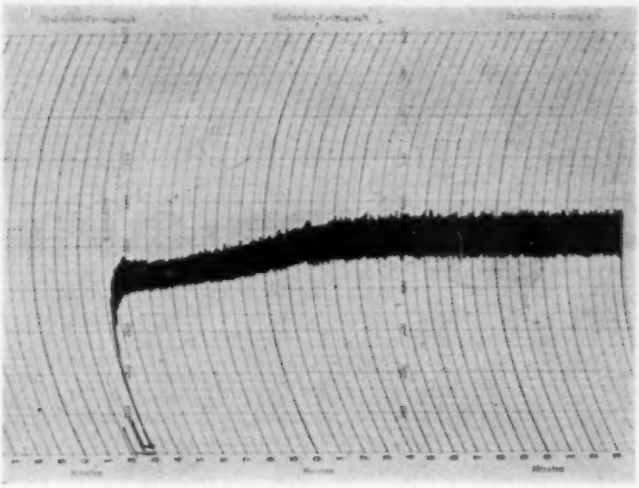

Fig. 8. Farinograph curve of a straight dough containing 0.5% of cuprous chloride (flour basis) mixed in oxygen before fermentation. (This curve is comparable to those in Figure 1; the same flour, formula, and procedure were used for the curves in Figures 1 and 8.)

in order to inactivate the enzyme, then used in a dough which was mixed in air; all the mixing was done in the farinograph. The curves obtained in these tests are shown in Figure 9.

Analysis of Figure 9 indicates that quinua must have at least several times the concentration of oxidizing enzyme as have American bread flours. The dough containing as little as 10% quinua flour, when mixed in air, showed a typical oxygen effect and, when mixed in nitrogen, was similar to the control without quinua flour which was mixed in air.[5] The dough containing 10% quinua flour that had been heated to inactivate the enzyme was also similar to the control, in that it did

[3] See anonymous article entitled "Bolivia to use quinua flour" in Modern Miller and Bakers' News 73 (51): 16. Dec. 21, 1946.

[4] See footnote 6.

[5] The curve for the quinua dough that was mixed in nitrogen before fermentation was as good in development as the control curve; this may indicate that the oxygen adsorbed or held mechanically by the flour was sufficient to produce some effect.

not show an oxygen effect when mixed in air.[6] Hence, the activity of the system was nullified either by depriving it of the oxygen in the air or by denaturing it with heat.

Discussion

The role of oxygen in dough mixing is of fundamental importance, but apparently this has not been fully appreciated in the past. Undoubtedly, the good effects attributed to high-speed mixing were actually largely due to the incorporation of increased amounts of oxygen, as compared to low-speed mixing. The essential function of oxygen in dough mixing presupposes a definite mechanism for its utilization in the gaseous or molecular form. The mechanism is apparently enzymic in nature.

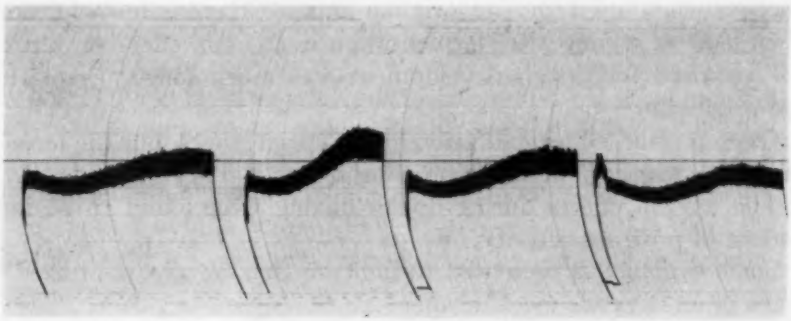

Fig. 9. Farinograph curves of straight doughs containing quinua flour. Reading from left to right the treatments of the doughs were as follows:
Control (no quinua flour); dough mixed in air before fermentation.
10% quinua flour; dough mixed in air before fermentation.
10% quinua flour; dough mixed in nitrogen before fermentation.
10% quinua flour which was first heated to 95°C, then used in dough which was mixed in air before fermentation.

According to Jørgensen (1945) the beneficial effects of oxidation in dough are due to protease inhibition. Certain workers have indicated that this theory may be unable to explain the effects of oxidizing agents in dough made from patent or bakers grade flour. Laitinen and Sullivan (1941) and Baker, Parker, and Mize (1942) have placed emphasis on a probable effect on the gluten as an alternative explanation. In mixing, the action of oxygen is immediate and seems to affect the gluten. The reaction is apparently unrelated to protease activity. From this it may be inferred that the effects of oxidizing agents are similar in character. The protease theory may be operative to the

[6] The fact that the curve for the dough containing heated quinua indicated slightly lower consistency than did the control curve, although paralleling it in shape, may be explained by the additional water which was required by the former dough due to effects produced during heating, such as gelatinization of starch in the quinua flour; in making this adjustment, too much water was added, producing the lower consistency.

extent that protease is active in the dough, but since the protease content of American bread flours appears to be negligible, the theory must assume a minor role in explaining oxidation effects in doughs made from such flours.

The essential nature of oxygen in dough mixing may lead to practical application in the baking industry, since oxygen produces improvements in dough and bread quality beyond the results obtained by mixing in air.

Summary

Mixing studies in which both straight and sponge doughs were mixed in the presence and absence of oxygen were made with the aid of the farinograph and general breadmaking procedures.

Oxygen is essential to proper dough development and was most effective when used in the original mixing (before fermentation). When used in mixing after fermentation it was still effective, but the time required for dough development was much longer than when used originally.

Oxygen retarded fermentation in the initial stages, but the fermentation rate was normal in the proofing stage.

The oxygen effects during dough mixing were found to be independent of protease activity.

Some evidence is presented to indicate that oxygen is utilized by means of an enzymic mechanism.

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EFFECT OF EXCESSIVE METHYL BROMIDE FUMIGATION ON FLOUR[1]

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(Received for publication May 12, 1947; presented at the Annual Meeting, May 1947)

Control of insect pests in food-processing plants, storehouses, and in stored products by means of fumigation is a very widely accepted practice. Elimination of these pests in the food industries is a matter of considerable importance from an economic as well as a public health standpoint. Successful fumigation practice involves the effective destruction of insects without leaving toxic or undesirable residues in the fumigated product.

Among the many commercial fumigants used, methyl bromide is one of the most recently developed. The use of methyl bromide has increased tremendously within the past few years because it can be easily handled and is extremely toxic to insect life. Several investigations have demonstrated that there are no harmful effects from methyl bromide residues in foodstuffs. However, complaints occasionally do occur regarding the unpleasant odor and taste of products baked from flour fumigated with methyl bromide. It seems apparent that these alterations occur when flour becomes exposed to excessive amounts of fumigant.

The object of this research was to determine the nature of the changes in flour fumigated with methyl bromide and particularly to investigate the factors responsible for the development of undesirable odor and taste. Further, it was desired to study the effect of moisture on the sorption (combined adsorption and absorption) of methyl bromide, as well as any alterations of the physical properties of dough subsequent to fumigation.

To date most of the papers on methyl bromide deal with the techniques of commercial fumigation. However, such topics as the rate of sorption during fumigation, toxicity to laboratory insects, and the effects of bromide residues in foodstuffs on laboratory animals have been investigated. Roehm, Shrader, and Stenger (1942) have determined bromide residues in cereals fumigated with methyl bromide.

Mackie and Carter (1937) presented some of the first evidence in favor of the use of methyl bromide in the fumigation of infested fresh vegetables. They claimed that in using normal dosages the vege-

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[1] This paper represents a portion of a thesis presented to the Graduate School of Kansas State College in partial fulfillment of the requirements for the degree of Master of Science.

[2] Present address: Armour and Company, Chicago 9, Illinois.

[3] Contribution No. 138, Department of Milling Industry.

tables were not damaged. On the other hand, Phillips, Munro, and Allen (1939) found that while methyl bromide is practical in the destruction of insects in harvested apples, under certain conditions the treatment results in both external and internal injury to the fruit. Dudley *et al.* (1940, 1942) found that dried fruits, fresh fruits, and vegetables absorbed minor quantities of the fumigant; but milled grains, cheese, nuts, and nut meats absorbed greater amounts. These authors believed that milled grains sorbed more methyl bromide because of their greater surface area, while the oily and fatty foods sorbed large quantities of the fumigant because of its solubility in fats.

Shepard and Buzicky (1939) reported that baking tests with flour fumigated with methyl bromide at two pounds per 1,000 cubic feet showed no detectable injury. Searls (1943) stated that methyl bromide produced a disagreeable odor on some furs and leathers, but there are no reports in the literature describing the odoriferous reaction of this fumigant with flour.

Materials and Methods

Preliminary studies were carried out with a bleached and malted commercial flour which had a protein content of 12.0%. Fumigations were performed at 25°C with a laboratory scale, fan-equipped, fumigator having a capacity of 116 liters (4.1 cubic feet). The dosage applied in most of the experimental fumigations was at a very high level of 25 pounds per 1,000 cubic feet for 24 hours, to insure overfumigation. This dosage is 12 to 25 times greater than is used in normal practice. For certain studies methyl chloride at similar dosage levels was also used.

Two years of extensive investigation in this laboratory on the effect of various amounts of methyl bromide applied to flour, on the development of odors in bread or toast made from the flour, demonstrated that several times the normal amount of fumigant are required to produce objectionable odors. When the fumigant is applied at the recommended concentrations of from one to two pounds per 1,000 cubic feet, or in slight excess of this amount, no odor developed after the flour was fumigated and aerated. In this investigation excessively heavy overdosages of methyl bromide were used to produce pronounced effects of overfumigation.

For the study of the relation of methyl bromide sorption to the moisture content of the flour, flours were exposed to different humidities until the desired moisture content was obtained. The samples were then placed in sacks and fumigated. Immediately after fumigation, each sample was thoroughly blended in a sealed mixer. One part was then set aside in a sealed container and the other part was

aerated for seven days at room temperature and then stored in sealed containers. A duplicate sample was allowed to aerate for seven days at room temperature before blending and storing in sealed containers.

Petroleum ether extracts were prepared by extracting flour with several portions of Skellysolve F at room temperature. The extracts were concentrated under vacuum at the same temperature.

The extracted flour was made into a dough using the appropriate amount of water, and fractionated into starch, gluten, and water-soluble material according to the technique described by Finney (1943).

The experiments reported in this work demonstrated that when fumigated flour is treated with a solution of potassium hydroxide or sodium hydroxide in ethyl alcohol, a characteristic, rather unpleasant odor is produced. This test was successfully employed to detect bromide residues due to chemical reactions in fumigated fractions of flour.

The Brabender Farinograph was used to determine the exact water absorption of the samples, to obtain doughs of the same consistency, and to indicate any alteration in the physical dough properties due to fumigation. Arbitrary values were given to the areas under the farinograms by means of the valorimeter as used by Johnson, Shellenberger, and Swanson (1946). The amount of sample employed in every test was corrected to 14% moisture basis.

The optimum dough mixing times were estimated with the Swanson-Working recording dough mixer, using the absorption found with the farinograph. This also permitted additional observations on physical dough properties.

The straight dough baking test method was employed. The formula and baking procedures used were those described by Johnson, Swanson, and Bayfield (1943).

The weight and volume of the loaves were determined immediately after they were taken from the oven. The bread was then placed in sealed cans, and on the following day was scored for grain, texture, exterior appearance, and odor. The odor rating was determined immediately after slicing the loaf. A panel of observers cooperated with this test. Also the original odor observations were followed by similar tests after toasting slices of the bread.

Several methods for determining small quantities of bromine in different materials have been published. In the present work the analytical procedure described by Shrader *et al.* (1942) for total bromide was used.

Gas production and gas retention were determined by means of the apparatus and procedure described by Working and Swanson (1946).

The estimation of the killing power of methyl bromide on the microflora of flour was carried out by the technique recommended by Kent-Jones and Amos (1930) and by Smith and Dawson (1944) for bacterial and fungal counts, respectively.

Results

Preliminary Investigations. Preliminary baking tests with flours fumigated at a dosage of 25 pounds per 1,000 cubic feet gave bread with a sharp, very objectionable odor. Upon cooling the loaves, the odor was not nearly so marked, but became strong again when slices of the bread were toasted. Flour fumigated with heavy dosages of methyl chloride produced baked loaves with a very objectionable odor but different from that given by methyl bromide fumigated flours. This experiment was tried as a side line to determine if a methyl halide other than methyl bromide would develop "off-odor" properties in bread.

In addition to the study of the effects of fumigation on flour it was planned to investigate the effects on the following four flour fractions: the petroleum ether extract, starch, gluten, and water-soluble material. However, early in the studies it was discovered by accident that 5% alcoholic potassium hydroxide (5 g. KOH in 100 ml. of EtOH), when added to fumigated flours, produced an unpleasant odor. The same reagent applied to unfumigated flour produced no odor. The application of this test showed that only the gluten and water-soluble fractions, i.e., the protein-containing fractions of flour, reacted with the fumigant; therefore it appeared unnecessary to consider all four fractions.

However, the petroleum ether extract from flour was at first included because according to Balls *et al.* (1940, 1942) it contained a lipoprotein with a high content of sulfur. It was thought that mercapto groups might perhaps be responsible for the odor obtained in the baked products of fumigated flours. Subsequently, however, a nitroprusside test demonstrated that nonfumigated flour, fumigated flour, fumigated flour treated with alcoholic potassium hydroxide, and an aqueous solution of methionine treated with liquid methyl bromide did not have free sulfhydryl groups.

Baking tests were made with nonfumigated flour, nonfumigated extracted flour, fumigated flour, and fumigated extracted flour. Both fumigated flours, extracted and unextracted, yielded products with the same unpleasant odor of approximately similar intensity. The odor was present in the loaves not only shortly after they were taken from the oven, but also on the following day after being stored in sealed cans.

Petroleum ether extracts from nonfumigated and fumigated flours

were treated with 95% ethanol in order to split the lipoprotein present in the extract. The two original extracts and the same extracts after ethanol treatment were fumigated with heavy dosages of methyl bromide. All samples gave a negative odor reaction when treated with alcoholic potassium hydroxide. On the other hand, gliadin and glutenin separated with 70% ethanol from gluten washed from fumigated flours both gave a positive odor test of approximately the same intensity with alcoholic potassium hydroxide.

When gluten was treated with methyl bromide and then added to flour it had a detrimental effect on the baking qualities as evidenced by a reduction in the loaf volume of the bread. This result was obtained by treating washed, dried, and finely ground gluten with liquid methyl bromide. After treatment the gluten was thoroughly aerated at room temperature until no evidence remained of the presence of the volatile fumigant. Two portions of the same flour were baked, one containing a 5% addition of treated gluten, the other an equal quantity of untreated gluten. The results were as follows:

Material	*Loaf volume, cc.*
Flour (check)	745
Flour plus 5% gluten	857
Flour plus 5% treated gluten	610

When an attempt was made to wash out gluten from flour treated with liquid methyl bromide, a glue-like substance was obtained. Gluten from fumigated flours also shows alteration, but not to such a marked degree.

Miscellaneous Materials Fumigated with Methyl Bromide. Twelve different materials were fumigated with methyl bromide at a dosage of 25 pounds per 1,000 cubic feet. These materials were corn flour, rye flour, oat flour, flax flour, barley malt, navy beans, soya beans, sorghum, dried gluten, dried blood fibrin, egg albumin, and gelatin. The alcoholic potassium hydroxide odor test gave positive results for all but gelatin. Thus gelatin was the only material that did not give the characteristic odor with the reagent.

Influence of Moisture Content on Sorption of Methyl Bromide by Flour. Flours ranging in moisture content from 9 to 16% were studied for bromide residues after similar methyl bromide fumigation. The extent of bromide sorption after fumigation and aeration at each moisture level is shown in Figure 1. The moisture contents after fumigation agreed within 0.1% of the values before fumigation. Bromide values are expressed in terms of the bromide ion.

The results shown in Figure 1 indicate that at moisture contents higher than 13.7% the sorption of bromide increases with the moisture

content of the flour. The correlation coefficient between the apparent bromide sorbed and moisture content of the flour is $r = 0.97$ (see Figure 2). At flour moisture levels above 15% the bromide sorbed during fumigation was almost completely retained even after aeration.

Several factors appear to control the sorption of methyl bromide by flour. Gases can diffuse into flour through the free spaces between the flour particles. Water vapor present in the atmosphere of the void spaces hinders diffusion of gases and at the same time it forms a film around the particles. To a great extent, such water films probably control sorption of the fumigant. This would account for the high

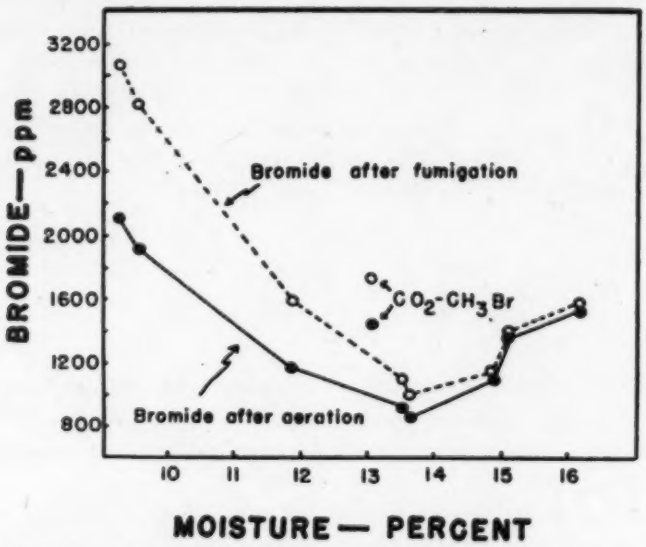

Fig. 1. The relationship between moisture content of flour and bromide sorbed after fumigation. The effect of aeration on bromide sorbed is shown. The results of one fumigation with a mixture of carbon dioxide–methyl bromide is indicated by two points at the 13% flour moisture level.

sorption of methyl bromide, and the comparatively low retention after aeration, in flours containing less than 13.7% moisture. It might be assumed that retention is primarily due to chemical reactions between methyl bromide and proteins and only secondarily to hydrolysis of the fumigant.

When moisture increases, the sorption of the fumigant during fumigation is hindered, but retention is higher due to greater hydrolysis. After a moisture content of about 13.7% is reached, the sorption due to hydrolysis seems to prevail.

Farinograms and mixograms were made of all samples (Figure 4). Table I shows the absorption determined with the farinograph and the values found with the valorimeter from the farinograms. Discussions of the influence of amino acids and carbon dioxide–methyl bromide

fumigated samples appear later. All of the curves for the treated flours had shorter mixing times and more rapid decreases in dough consistency than did the curve of the check flour. The valorimeter readings for the fumigated flours are lower than for the control. It is evident that fumigation caused changes in the flours which altered the physical properties of the doughs. The kind and degree of the alterations seem to be regulated to a great extent by the moisture content of the flour. Thus, samples 7 and 8 (high moisture flours) yielded narrower tracings at the end of the farinograms than did the other samples. They also showed lower consistency at the end of the mixograms than the other fumigated samples.

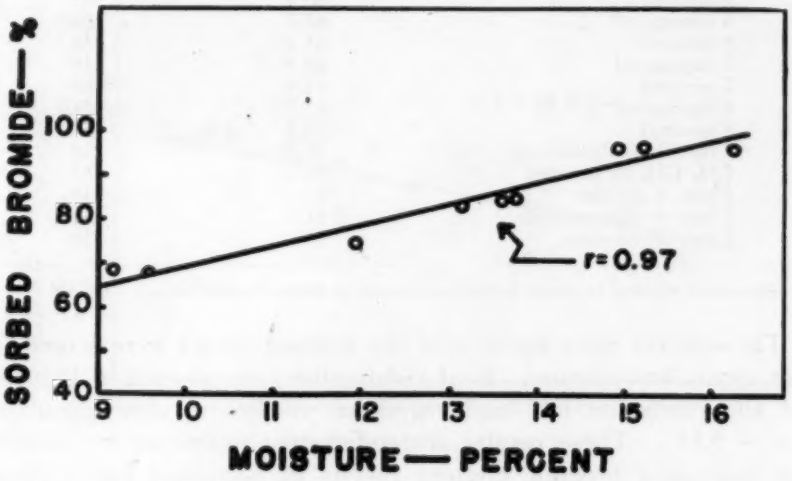

Fig. 2. Correlation between flour moisture content and sorbed bromide retained after aeration.

The hydrogen-ion activity of the fumigated flours was determined. Typical values are as follows:

Sample	*pH value*
Control	5.8
1b (dry; high bromide residue)	5.4
4b (normal; lowest bromide residue)	5.6
8b (wet; medium bromide residue)	5.4

From these typical results it appears probable that the hydrogen-ion activity bears some relation to the bromide residue in the samples. However, the whole picture appears to be a balance between the chemical action of methyl bromide on the proteins; the effect of moisture on the hydrolysis of the fumigant; the effect of hydrolytic products on the flour properties; and the influence of the higher acidity due to the presence of these products.

TABLE I

FLOUR ABSORPTIONS AND VALORIMETER READINGS DETERMINED WITH THE FARINOGRAPH ON FUMIGATED AND AERATED SAMPLES

Sample no.	Absorption[1] (%)	Valorimeter reading
Control	61.1	63
1 fumigated	61.3	60
1 aerated	61.0	62
2 fumigated	59.8	55
2 aerated	59.9	59
3 fumigated	60.5	60
3 aerated	61.0	62
4 fumigated	61.3	62
4 aerated	61.5	62
5 fumigated	60.4	61
5 aerated	60.4	62
6 fumigated	61.3	60
6 aerated	61.4	58
7 fumigated	61.9	59
7 aerated	61.9	60
8 fumigated	62.2	58
8 aerated	61.2	53
CO_2–CH_3Br fumigated	60.8	62
CO_2–CH_3 Br aerated	60.5	65
Flour + cystine	61.1	56
Flour + tryptophane	61.1	58
Flour + tyrosine	61.1	56

[1] Absorption required to obtain a dough consistency of 500 Brabender units.

The samples were baked and the finished loaves were scored for odor, grain, and texture. Loaf volume data are plotted in Figure 3 and the regression line and regression coefficient were calculated ($r = -0.85$). These results are sufficiently significant to indicate that increasing bromide content results in decreased loaf volume. Samples with relatively low bromide content had a less intense odor than the other samples, and the samples with the least bromide residue showed the best grain and texture.

Fumigation with Carbon Dioxide–Methyl Bromide. It was thought that it might be possible to use some other gas to react selectively with the proteins of flour and thus prevent methyl bromide from reacting. Carbon dioxide was tried in an experiment because its use in mixtures with other fumigants had been recommended.

Results for bromide residues are shown in Figure 1 as two isolated points. It should be noted that the values obtained are higher than the corresponding values, at similar moisture content, when bromide is used alone. But Figure 1 shows that the percentage of bromide sorbed in fumigation that was retained after aeration agreed with the values obtained with methyl bromide alone.

The addition of carbon dioxide to the fumigant does not appear to reduce the sorption of methyl bromide; probably this inertness

nullifies its use to prevent alterations of a chemical nature with methyl bromide. The farinograms obtained with these samples are shown in Figure 4.

Addition of Amino Acids. It is probable that reactions of methyl bromide with the flour proteins are dependent on the presence of a specific group in the molecules of amino acids, or on a specific amino acid itself. In order to throw some light on the latter assumption, 0.1% cystine, 0.1% tryptophane, and 0.05% of a crude preparation

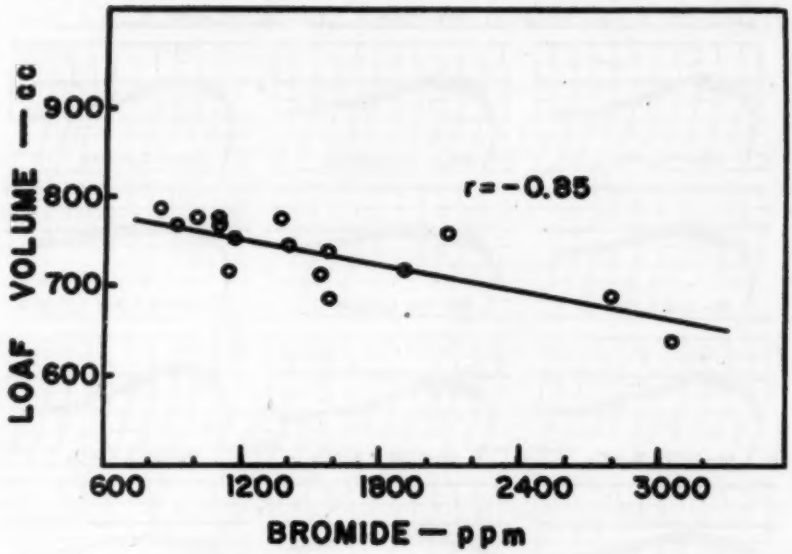

Fig. 3. Correlation between loaf volume and bromide sorbed.

of tyrosine were added to three samples of flour (13.3% moisture). They were then fumigated in the usual manner. The farinograms shown in Figure 4 do not indicate greater alterations than were obtained with fumigated flour.

Aqueous solutions of methionine and tryptophane were treated with liquid methyl bromide, the solutions added to nonfumigated flours, and the flours baked. Methionine-treated flour gave loaves with a very strong objectionable odor, while the tryptophane-treated flour yielded normal bread. But the same odor obtained with treated methionine was produced when a plain water solution of the amino acid was used. It is evident that the objectionable odor in this experiment was due to decomposition of methionine, and not to methyl bromide action.

Treatment of Fumigated Flours with Oxidizing Agents. Farinograms and mixograms prepared with fumigated flours resembled those

obtained with the addition of reducing substances like cysteine. For this reason the action of oxidizing agents on the pattern of the curves was determined. Sample 8 (Table I) was used for these experiments.

Farinograph curves were made with the appropriate amount of sample, and with added iodine, potassium bromate, and potassium

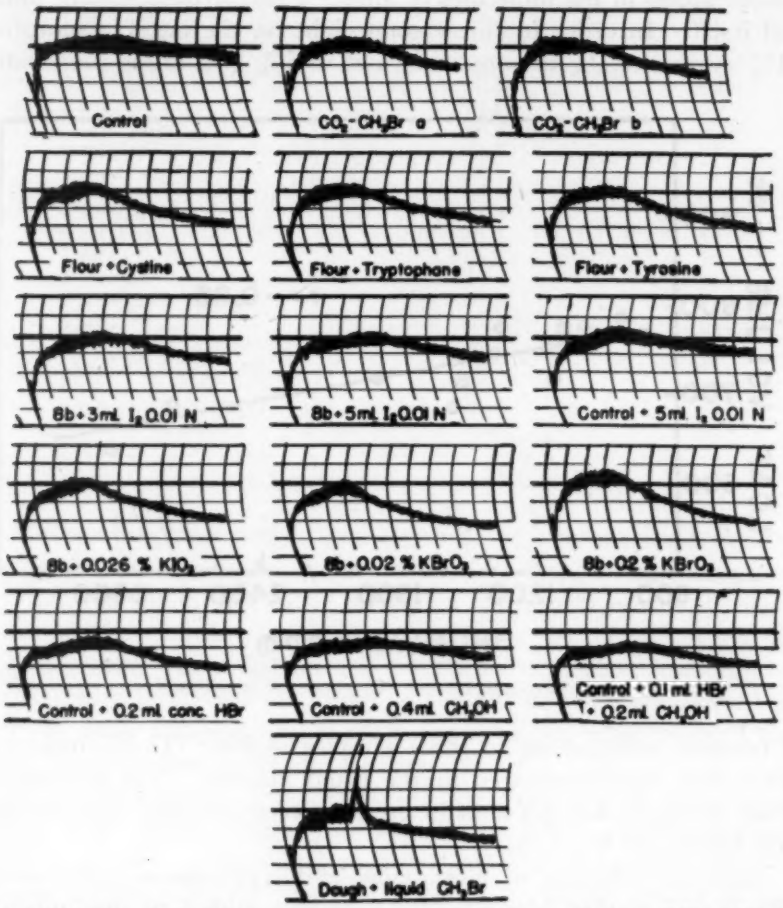

Fig. 4. Changes in the farinogram patterns produced by various substances. Each vertical line equals two minutes. The heavy horizontal line equals 500 Brabender units. CO_2–CH_2Br a and CO_2–CH_2Br b represent the same flour sample which contained 13.1% moisture and retained 0.17% and 0.14% Br before and after aeration. The curves shown in the bottom row were obtained when the reagents indicated were added to the water used in the farinograph.

iodate. The curves obtained for these variously treated fumigated flours are presented in Figure 4. Addition of 3 ml. of iodine in potassium iodide (0.01 *N*) resulted in a curve with good recovery. A better recovery was obtained after using 5 ml. of the same solution. On the other hand, 0.026% of potassium iodate and either 0.02% or 0.2% of potassium bromate did not show satisfactory improvement.

The favorable effect obtained with iodine solution might be due to the slight stiffening effect on the dough. In fact, nonfumigated flour treated with 5 ml. of the iodine solution showed that the dough had been toughened (Figure 4).

Treatment of Flour with Hydrobromic Acid and Methyl Alcohol. Since hydrobromic acid and methyl alcohol are possible hydrolytic products of methyl bromide, it seemed desirable to observe the effect of these compounds on flour. Hydrobromic acid produced a graph that resembles those obtained with fumigated flours. Methyl alcohol decreased the consistency of the dough but did not produce as fast a break as did methyl bromide. Incorporation of both agents together yielded an additive effect.

A nonfumigated flour treated with 0.5 ml. of concentrated hydrobromic acid and one treated with 0.5 ml. of methyl alcohol were baked according to the procedure already outlined. Neither one of these reagents produced loaves with objectionable odors, although they showed a detrimental effect on the volume. This was especially apparent with hydrobromic acid because it produced a decrease in loaf volume of 25%.

These results might suggest that methyl bromide has a twofold effect on flour. One is the specific reaction of the organic halide with a certain part or parts of the protein-containing fraction. The other is the action of the hydrolysis products, mainly hydrobromic acid, on the physical dough properties.

Treatment of Dough with Liquid Methyl Bromide. Reactions between methyl bromide and gluten proteins might occur at those parts of the molecules which are responsible for the formation of gluten. A study was therefore made to determine whether or not the fumigant could react with the proteins *after* the gluten network was established. Figure 4, bottom curve, shows the curve produced when the dough was treated with liquid methyl bromide just as it reached its peak consistency in the mixer. The sudden increase in consistency may be due to the cooling action of evaporation.

Gas Production and Gas Retention. It was of interest to determine whether or not bromine residues in methyl bromide fumigated flours affect the yeast used in baking and also if the ability of the gluten network to retain the carbon dioxide produced during fermentation is influenced. Measurements of the rate of gas production from a dough furnish information on the fermentation activity of the dough. Gas retention is useful to determine, roughly, alterations in the physical properties of the proteins. In the former determination the total volume of carbon dioxide produced during fermentation is measured, while in the latter only the gas retained in the dough is evaluated.

A hard winter wheat straight grade bakers' type flour with a moisture content of 13.9% was fumigated with methyl bromide at a dosage of 25 pounds per 1,000 cubic feet for 24 hours. The bromide sorbed after aeration was 0.086%. The fumigated sample gave a longer period of constant fermentation rate than the nonfumigated. The percentage of total gas produced which is retained by the dough showed a small decrease for fumigated flour, and in this respect confirms what was found by the baking tests.

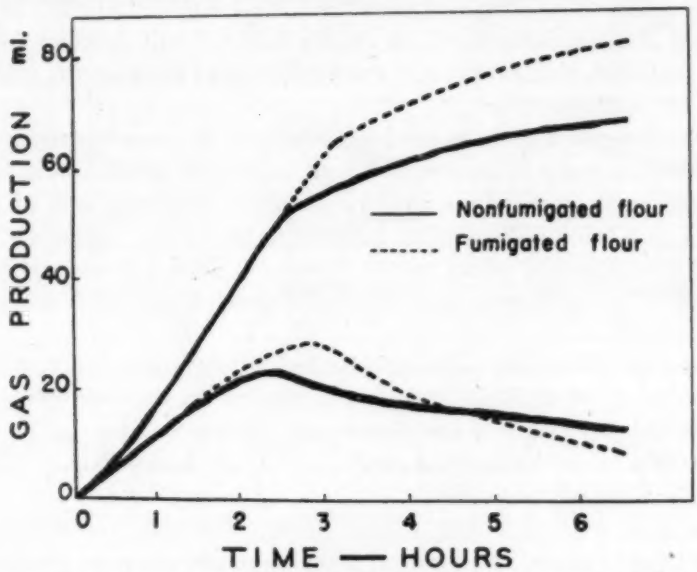

Fig. 5. The effect of excess methyl bromide fumigation on the gas production and gas retention properties of flour. Upper curves show gas production and lower curves show gas retention.

Selenium-Containing Flours. It is well known that selenium compounds tend to form products with very sharp odors. To determine whether a selenium-bearing flour showed more pronounced odors than a normal flour, a wheat containing 26.6 p.p.m. of selenium was milled, and two samples of the flour were fumigated with methyl bromide, one with 2 pounds per 1,000 cubic feet and the other with 25 pounds per 1,000 cubic feet. Both samples were baked. The odor of the loaves was no more objectionable than that of common flours fumigated at the same dosages.

Germicidal Effect of Methyl Bromide. During the course of this work it was found that flours fumigated with methyl bromide appeared to be relatively free from microflora. In order to corroborate this observation microfloral counts were made.

The results obtained, although of a preliminary nature, are considered important and significant. In these experiments four samples

of the same flour were used for assay of bacteria and fungi. Two of the samples had been fumigated with methyl bromide at dosages of 2 and 25 pounds per 1,000 cubic feet, another one had been treated with a similarly heavy dosage of methyl chloride, and the fourth was a nonfumigated control. The data obtained are presented in Table II and show that even a moderate dosage of fumigant had a pronounced microbicidal effect. Methyl chloride likewise appreciably decreased the number of microorganisms per gram.

TABLE II

MICROFLORAL COUNTS ON FUMIGATED FLOURS

Treatment of sample	Incubation: 4 days; dilution 1/100 microorganisms per gram	
	Nutrient agar	Rose Bengal[1]
Check	approx. 30,000[2]	1,700–2,700
Methyl bromide		
25 lbs./1,000 cu. ft.	0–100[2]	0–300
2 lbs./1,000 cu. ft.	200–400	0–100
Methyl chloride		
25 lbs./1,000 cu. ft.	700–1,200	100–300

[1] Rose Bengal medium preferentially inhibits bacteria growth.
[2] Incubation period: 2 days.

Discussion

All the evidence gathered during the various stages of this study indicates that methyl bromide reacts with the protein fractions of flours. Methyl chloride is also able to react with flour. However, the products of these two reactions apparently are different, because the odors of the loaves baked from the treated flours are unlike.

A reaction similar to that of methyl bromide on the proteins of flour occurs with other proteins. Of a number of proteins investigated, gelatin was the only one to give a negative reaction when exposed to methyl bromide and treated with alcoholic potassium hydroxide. Gelatin does not contain the amino acids valine, beta-hydroxyglutamic acid, tyrosine, methionine, and tryptophane. This deficiency may be significant as an indication that one or more of the amino acids missing in gelatin are responsible for the odor produced when methyl bromide reacts with protein.

The moisture content is important in the sorption of methyl bromide by flour, since it appears to control both the amount of fumigant fixed as well as its mode of action. When moisture is low, methyl bromide is bound mostly by chemical reactions with the protein fractions; conversely, when moisture is high it hinders these reactions and favors hydrolytic phenomena. The products of these two modes of action are different and the effects also are different. It is of par-

ticular significance that minimum sorption of the bromide takes place in the neighborhood of 14% moisture (Figure 1).

It is evident that the high bromide residues resulting from overfumigation decrease the baking quality of flour. This is true not only because of the objectionable odor that develops in the baked loaves but also because of alterations in the physical properties of the dough. Accordingly, methyl bromide fumigation of flour should be performed with considerable care to avoid overfumigation.

One of the objectives of this work was to find some means to avoid the bad effects of overfumigation. However, no way has been discovered to alleviate the damage to the flour once overfumigation occurs. Aeration, of course, helps to eliminate the volatile fumigant that has not reacted with the flour, but aeration does not change the amount of sorbed methyl bromide.

Further work should be directed toward determining the reaction between methyl bromide and cereal proteins. If the reaction were known it might be possible to correct the difficulty, although this research appears to indicate that the reaction is severe enough to alter the protein irreparably. It was thought that the fumigant might attack the disulfide linkages (–S–S–) and liberate sulfhydryl groups (–SH). This should weaken the protein structure, especially the cross linkages, and at the same time the sulfhydryl groups should contribute to the off-odors which appear in baking. The nitroprusside test, however, has shown that there is no liberation of sulfhydryl groups, even if the fumigant acts directly on an amino acid like cystine or methionine. The latter amino acid was chosen because, as previously noted, it is not present in gelatin. Further, the $-SCH_3$ group of this amino acid could conceivably react with methyl bromide to give an addition compound (sulfonium compound) which could be decomposed by heat. However, these experiments did not confirm this possibility. Furthermore, it was found that when cysteine, which contains a free –SH group, is used in baking it markedly affects the baking quality of flour but does not give an objectionable odor.

Tryptophane was another amino acid tested because of its absence in gelatin and because of its intimate relation with skatole and other indole derivatives which are usually very odoriferous. It also failed to give any reaction with the fumigant.

During the fermentation of doughs made up from nonfumigated flour the normal microflora may use part of the sugar present for their own metabolism. With destruction of the microflora this sugar would remain in the doughs and be available to the yeast. The longer maintenance of peak fermentation rate with the fumigated flour could therefore be explained in this manner. However, a more logical ex-

planation is on the basis of the stimulation of yeast activity. On the other hand, a change in the starch-amylase relationship in the dough due to methyl bromide fumigation is not excluded. No investigations have been made in this direction, but it is possible that the bromide reaction could result in either the starch being made more available to hydrolysis or the amylases become more active. In either case there would be a net increase in the amount of fermentable sugar.

Complete or partial elimination of the microorganisms present in flour would be of great value in preventing the development of "rope" in bread. In future studies with methyl bromide fumigation of flours it would be very desirable to determine whether or not flours fumigated at normal dosages can develop rope. Thus an important contribution to the baking industry might be made. Furthermore, methyl bromide could possibly be used as a tool for disinfection of materials when heat cannot be employed.

The effects of methyl bromide fumigation on flour properties therefore are twofold. If used in excessive amounts, it is detrimental to baking quality. On the other hand, in addition to serving as an insecticidal agent, it appears to have definite promise as an effective germicide.

Summary

The effect of excessive methyl bromide fumigation of flour on the physical properties of doughs, the quality of bread produced, and the differential effect of various flour constituents were investigated.

Fumigation with normal concentrations of methyl bromide (1 to 2 pounds per 1,000 cubic feet) causes no lasting deleterious effects on flour. However, at high concentrations such as used in this study (25 pounds per 1,000 cubic feet) irreversible damaging changes occur. Tests showed that the changes are associated with the gluten protein fractions of flour. Excessive methyl bromide treatment of flour reduces dough development time and lessens mixing tolerance, as indicated by dough development curves. The loaf volume of bread made from treated flour is reduced and the bread produced has an undesirable odor. The importance of avoiding the overfumigation of flour is apparent.

Moisture content was shown to be of primary importance in controlling the sorption of methyl bromide. At moisture values below 14% chemical action on proteins is involved, whereas at high moisture values the effects appear to be associated with hydrolytic products of the fumigant.

Methyl bromide appears to be an effective germicidal agent for flour.

Acknowledgments

The authors are indebted to the Dow Chemical Company for the research grant which made this work possible.

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A SIMPLE SEDIMENTATION TEST FOR ESTIMATING THE BREAD-BAKING AND GLUTEN QUALITIES OF WHEAT FLOUR

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A test for estimating potential bread-baking quality, that is more simple, rapid, and practical than the Kjeldahl protein test or other tests now used to evaluate wheat in terms of baking quality, would be extremely valuable for use in the grading of wheat. To be practicable for this purpose a test should be simple enough to be applied by grain inspectors who have not had chemical training, should be rapid enough not to delay grading seriously, should require no expensive or elaborate equipment, and should predict wheat quality at least as accurately as existing tests.

The conventional Kjeldahl protein test, used to evaluate wheat, does not meet these requirements for a practical test, particularly in respect to the simplicity of equipment needed and technical training necessary. Furthermore, the Kjeldahl protein test does not adequately reflect the bread-baking potentialities of wheat that has inferior gluten quality as a result of unfavorable environmental conditions during growth, damage in storage, or because it is of a variety having inherently inferior gluten quality.

It has long been known that differences among flours from different types of wheat are reflected by the abilities of the gluten proteins to imbibe water. The relationship between the colloidal swelling of gluten and the bread-baking quality of flour was probably first reported by Upson and Calvin (1916). Gortner and Doherty (1918), in studying the rate and extent of the swelling of gluten disks in dilute solutions of various acids, found that glutens from "strong" flours have much higher rates of hydration and much higher hydration capacities than do glutens from "weak" flours. Lüers and Ostwald (1920) and Gortner and Sharp (1923) demonstrated the relationship between flour baking strength and hydration capacity as measured by the viscosity of acidulated suspensions of flour in water. Lüers and Schneider (1921), in comparing various methods for determining the hydration capacity of colloids, found good agreement among Hofmeister's method of weighing before and after the imbibition of water by the colloid, Fischer's method of determining the change in volume, and the viscosity method.

[1] Chief, Standardization Research and Testing Division.

Numerous investigations have been made in recent years on the viscosity of flour-water suspensions, and viscosity measurements have been found useful in evaluating soft wheat flours. Finney and Yamazaki (1946), in applying viscosity measurements to hard wheat flours, found that for individual varieties there is an essentially linear relationship between viscosity and protein content, but that this relationship is not the same for different varieties and that these varietal differences cannot be properly evaluated by means of viscosity measurements.

Finney and Yamazaki (1946), in further investigations, however, developed a method of testing by which the water-retention capacity of hard wheat flour is measured by determining the weight of material separated from an acidulated flour-water suspension by means of centrifugal force under prescribed conditions. The values obtained correlated well with bread loaf volumes and appeared to evaluate the various varieties of hard red winter wheat properly in terms of their gluten quality.

The United States Department of Agriculture is carrying on several lines of research in an effort to devise a suitable practical test that may be used in connection with the official inspection of wheat and that will reflect with at least reasonable reliability the baking quality of the flour that can be milled from the wheat. This is a preliminary report of a somewhat novel approach to the problem which has progressed far enough to be of general interest. The test described is based on the rate of sedimentation of the solid phase from an acidulated suspension of flour in water. The test has so far been applied only to white flour, but it is anticipated that further research will result in its application either to whole wheat meal or to a crude white flour quickly prepared without elaborate milling.

Method

In an effort to adapt measurements of water imbibition to a procedure that would meet the previously mentioned requirements of a practical grain inspection test, it was noted that the rate at which the solid phase of an acidulated water suspension of whole wheat meal settles to the bottom of a container varies greatly among different samples of wheat. Rapid settling of such suspensions was found to be associated with low protein content and with wheat varieties having poor gluten quality. Conversely, slow settling was observed to be associated with high protein content and good gluten quality. The solid phase of this type of suspension appears to consist of a mass of greatly swollen gluten particles in which are imbedded most of the other insoluble constituents of the wheat. The level to which the solid phase will settle under the

force of gravity in a given interval of time depends largely on the quantity of swollen gluten present and on the degree of swelling. The greater the amount of water imbibed by the gluten the lower will be the specific gravity of the swollen gluten and the slower will be the rate at which it will settle. Attempts to apply this principle to the testing of whole wheat meal immediately brought to light the fact that variations in the method of grinding the wheat may greatly affect the results obtained. In order to eliminate as far as possible this variable factor until the possible usefulness of the principle itself could be further investigated, the present studies have been confined exclusively to experimentally milled, unbleached, unenriched white flour.

The following procedure was applied to all the flour samples tested:

> Place a quantity of flour equivalent to 4.00 g on a 14% moisture basis in a 100 ml glass-stoppered graduated cylinder having a distance of from 180 to 185 mm between the zero and 100 ml marks. Add 50 ml of distilled water to the cylinder, shake the mixture for 30 seconds, and allow it to stand for 5 minutes. Add 25 ml of dilute lactic acid,[2] then mix the contents of the cylinder by inverting the stoppered cylinder and returning it to the upright position 10 times. (Do not shake the cylinder.) Immediately after mixing place the cylinder in an upright position and start timing with a stopwatch or interval timer. After an interval of exactly 5 minutes read the volume of the solid phase of the material in the graduate. This volume in milliliters is the "sedimentation value" of the flour.

Figure 1 shows the appearance of the sedimentation tubes at the time readings are taken. The line of demarcation between the solid and liquid phases is ordinarily sharp and distinct, as shown in the figure, so that readings may be made to the nearest milliliter and estimated to the nearest 0.1 ml. Occasionally the line of demarcation is less distinct but rarely is it so indistinct that readings may not be made to the nearest milliliter. Duplicate determinations usually agree within less than 1 ml. In a series of 135 samples of flour tested in duplicate, the average difference between duplicates was 0.5 ml and the maximum difference was 2.5 ml. Temperature of reagents within the range of 20°C to 30°C has little effect on the results. Minor variations in strength of acid or in the time and manner of shaking or mixing have no appreciable effect on the results. The 5-minute period of settling, however, must be accurately timed, since at that time sedimentation is still progressing fairly rapidly. Readings taken at longer time intervals show smaller differences between good and poor bread flours, and readings taken at shorter time intervals tend to be somewhat erratic. Sedimentation values can be expected to range from 20 or less for low protein flour of very inferior bread-baking

[2] The dilute lactic acid is prepared by diluting 250 ml of 85% lactic acid to 1000 ml. The diluted acid must be allowed to stand for at least 3 weeks before use, *or* refluxed at its boiling temperature for 6 hours without loss of volume and cooled to room temperature before use. The reagent thus prepared will keep indefinitely without change in strength.

quality to 55 or more for high protein flour of superior bread-baking quality. For comparative purposes the test should be applied to the same grade of flour (straight, long patent, short patent, etc.) since differences in flour ash content have some effect on the sedimentation values.

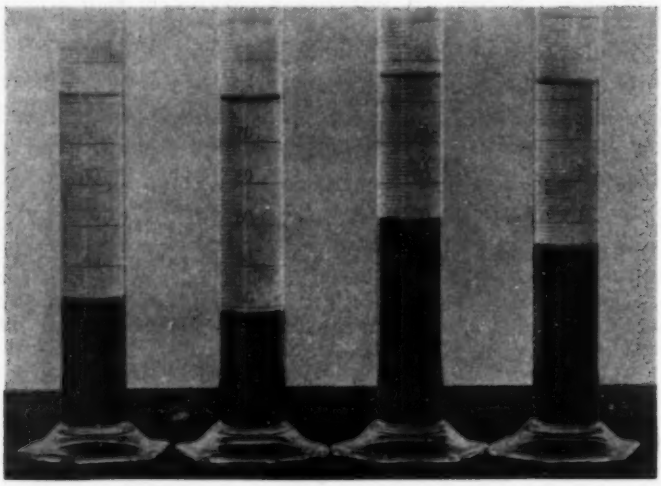

Fig. 1. Appearance of sedimentation tubes at the time of reading.

Materials

The sedimentation test was applied to 135 samples of hard wheat flour that had previously been milled and baked in connection with other research projects. These flour samples were milled experimentally from 52 samples of hard red winter wheat of known pure varieties grown in experimental plots at five stations in four states, six samples of commercially grown hard red winter wheat of known variety, 59 samples of commercial hard red winter wheat consisting mostly of mixtures of varieties that were tentatively identified from the physical characteristics of the kernels, and 18 samples of hard red spring wheat of known varieties grown in experimental plots at two stations.

The wheats were milled to 90% patent flours on the Allis-Chalmers experimental flour mill. The average ash content of the flours was about 0.42% (14% moisture basis). The bread-baking tests were made by a formula described by Fifield *et al.* (1945) using 100 g of flour, 2.0 g of compressed yeast, 1.5 g of salt, 5.0 g of sugar, 0.25 g of malted wheat flour, 3.0 g of shortening, 4.0 g of nonfat dry milk solids, and varying amounts (0 to 4 mg) of potassium bromate. The ingredients for two loaves were mixed together for a sufficient length of time for proper dough development by using a Hobart-Swanson dough

mixer with four pins in the head and two pins in the bowl and operated at 108 r.p.m. The doughs after mixing were divided into two equal parts, fermented for 3 hours at 30°C, panned and proofed for 55 minutes at 30°C, then baked for 25 minutes at 232°C. For the purposes of this study the loaf volume data in each instance is that of the loaf containing the amount of potassium bromate that produced the greatest loaf volume. In most instances the loaf having the greatest volume also had the best grain, texture, and crumb color.

Relation Between Sedimentation Time, Loaf Volume, and Protein Content

Average data for loaf volume, protein content, and sedimentation value of the 135 flour samples classified according to variety or type are given in Table I. The relationships between protein content and

TABLE I

AVERAGE LOAF VOLUME, PROTEIN CONTENT, AND SEDIMENTATION DATA FOR 135 SAMPLES OF HARD WHEAT FLOUR

Variety or type	No. of samples[1]	Average loaf volume	Average protein content[2]	Average sedimentation value	Average specific loaf volume	Average specific sedimentation
		ml	%	*ml*	*ml*	*ml*
Turkey (pure variety)	7	720	11.7	39.2	48.4	3.3
Wichita (pure variety)	6	748	12.3	41.4	48.5	3.3
Tenmarq (pure variety)	7	738	11.9	38.7	48.6	3.2
Pawnee (pure variety)	7	752	12.5	38.9	47.9	3.1
Comanche (pure variety)	7	743	12.4	44.4	46.9	3.6
Early Blackhull (pure variety)	5	670	11.6	35.3	45.0	3.0
Blackhull (pure variety)	6	706	12.3	35.9	44.9	2.9
Red Chief (pure variety)	7	599	11.6	29.2	37.7	2.5
Chiefkan (pure variety)	6	625	12.4	29.2	37.5	2.4
Commercial HRW (predominantly "desirable" varieties)	49	641	10.4	33.2	46.3	3.2
Commercial HRW (predominantly Red Chief or Chiefkan)	10	538	9.7	26.4	39.1	2.7
Hard red spring wheat [3]	18	819	12.6	43.9	52.2	3.5

[1] In the instance of each of the named pure varieties each sample was grown at a different station.
[2] Calculated to a 14% moisture basis.
[3] Pure varieties grown at two different stations.

loaf volume and between sedimentation value and loaf volume are shown graphically by means of scatter diagrams and regression lines in Figures 2 and 3 respectively.

The values obtained from statistical analysis of the data are given in Table II. From the correlation coefficients and standard errors of estimate it is evident that for this particular series of flour samples sedimentation rate is fully as good as protein content as an index of

bread loaf volume. From the scatter diagram in Figure 2 it may readily be seen that if the regression equation were to be used to predict loaf volume from protein content, the loaf volumes predicted for flour milled from Chiefkan or Red Chief wheat (varieties of generally recognized inferior gluten quality) would be expected to be consistently greater than the loaf volumes actually attained and the errors of prediction would in some instances be very large. Referring then to Figure 3, it is obvious that by using the sedimentation value the loaf volume of flour from Chiefkan and Red Chief wheat can be predicted on the same basis and with essentially the same degree of reliability

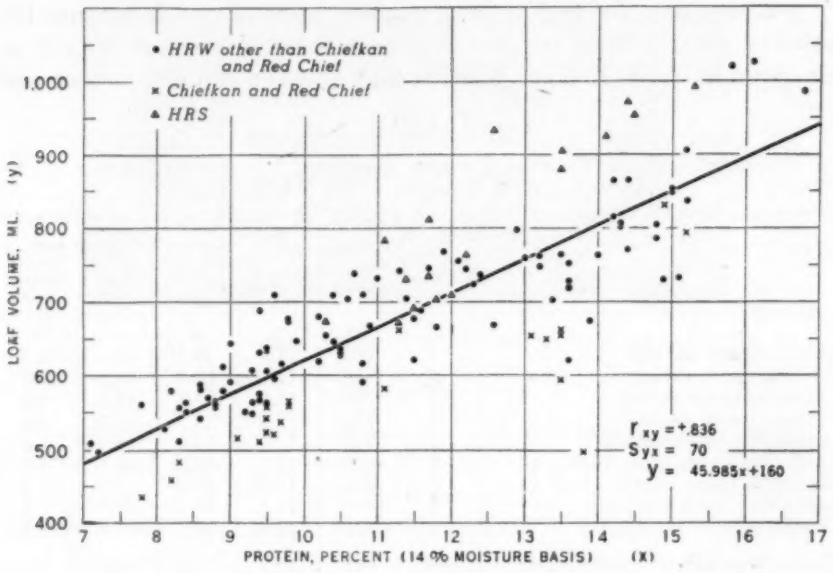

Fig. 2. Relation between protein content (14% moisture basis) and bread loaf volume.

as that of bread from other varieties of hard wheat. The sedimentation value tends to evaluate flour from Chiefkan and Red Chief wheat properly in comparison with flour from other varieties of hard wheat, while the protein test, on the other hand, almost invariably fails to reflect the relatively poor bread-baking quality of flour from these two varieties of wheat.

In the series of 135 samples of flour under investigation 17% of the samples consisted of flour milled from wheat entirely or predominantly of varieties of recognized inferior gluten quality. It is reasonable to assume that the greater the percentage (up to 50%) of such inferior gluten quality samples in a series, the lower would be the correlation between protein content and loaf volume for the entire series and

the greater would be the advantage of sedimentation value over protein content as a measure of loaf volume.

TABLE II

STATISTICAL ANALYSIS OF DATA

Correlation coefficients [1]	Standard errors of estimate	Coefficients of partial correlation
$r_{pv} = .836$	$S_{vp} = 70$ ml.	$r_{pv.s} = .497$
$r_{sv} = .863$	$S_{vs} = 64$ ml.	$r_{sv.p} = .604$
$r_{sp} = .790$	$S_{ps} = 1.42\%$	$r_{sp.v} = .249$

[1] p = percent protein, s = sedimentation value, v = bread loaf volume in milliliters.

The coefficient of partial correlation $r_{sv.p}$, having a value of .604 and showing the relationship between sedimentation value and loaf

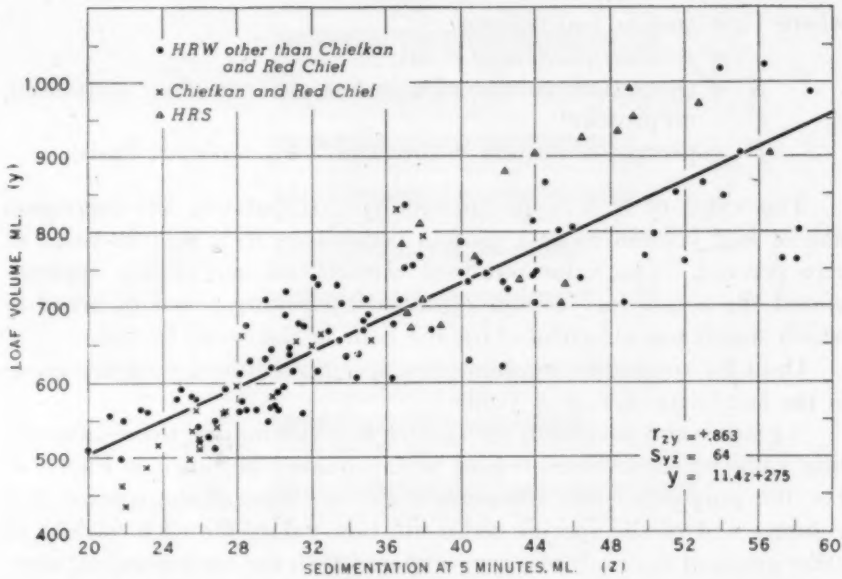

Fig. 3. Relation between sedimentation value and bread loaf volume.

volume independent of protein content, indicates that at a constant protein content level sedimentation value is significantly correlated with loaf volume. Then since differences in loaf volume at any protein content level are assumed to be caused by differences in gluten quality, the observation that sedimentation value is partially and significantly dependent upon gluten quality is supported by statistical evidence.

Specific Sedimentation as a Measure of Gluten Quality

Since the sedimentation value depends both on the quantity and quality of the gluten in the flour it should be a better index of potential

bread loaf volume than a test which measures only gluten quantity or gluten quality. In order to express the results of the sedimentation test in terms that tend to reflect only gluten quality, however, the sedimentation value in each instance was divided by the percentage of protein (on a 14% moisture basis) in the flour and the resulting value, for convenience, is called the "specific sedimentation."

Loaf volume, likewise, depends both on the quantity and quality of the gluten in the flour. In order to express loaf volume data in terms that tend to reflect only gluten quality, a value known for the purposes of this study as "specific loaf volume" was calculated by the formula:

$$V_s = \frac{V - K}{P}$$

where V_s = specific loaf volume
V = actual loaf volume in ml
K = theoretical volume of a loaf made from flour containing no protein
P = percent of protein in the flour (14% moisture basis).

The value of K was determined by extrapolating the regression line of loaf volume against protein percentage to a protein value of zero percent. The value obtained was 160 ml and closely approximated the actual loaf volume obtained by baking a loaf of bread in which starch was substituted for the flour in the bread formula.

Data for specific loaf volume and specific sedimentation are given in the last two columns of Table I.

A graphic comparison of the specific loaf volume and the sedimentation value methods of expressing gluten quality is shown in Figure 3. For the purpose of this comparison the averages of the specific loaf volumes and of the specific sedimentation values for each variety or other group of flour samples are compared with the corresponding average values for the 45 samples of flour from experimentally grown hard red winter wheats of known "desirable" varieties, which values are both arbitrarily taken to be 100. It is interesting to note that the individual varieties of hard red winter wheat, with the exception of the variety Comanche, are rated in essentially the same order by both methods of estimating gluten quality. The generally recognized marked inferiority of the gluten of Chiefkan and Red Chief wheat is fully reflected both by the specific loaf volume and specific sedimentation values. The sedimentation test appears to indicate that the gluten from Comanche flour is rather distinctly superior to that from any of the other "desirable" varieties of hard red winter wheat, a finding that is not confirmed by the baking tests. This suggests the possibility that flour

from Comanche wheat may have a bread-baking potentiality not fully reflected by our usual baking tests.[3]

The 23 samples of flour from wheat consisting entirely or primarily of the varieties Chiefkan or Red Chief were found without exception to have specific sedimentation values of less than 3.0. Of the 112 samples of flour from wheat consisting entirely or primarily of varieties other than Chiefkan or Red Chief, 91 were found to have specific sedimen-

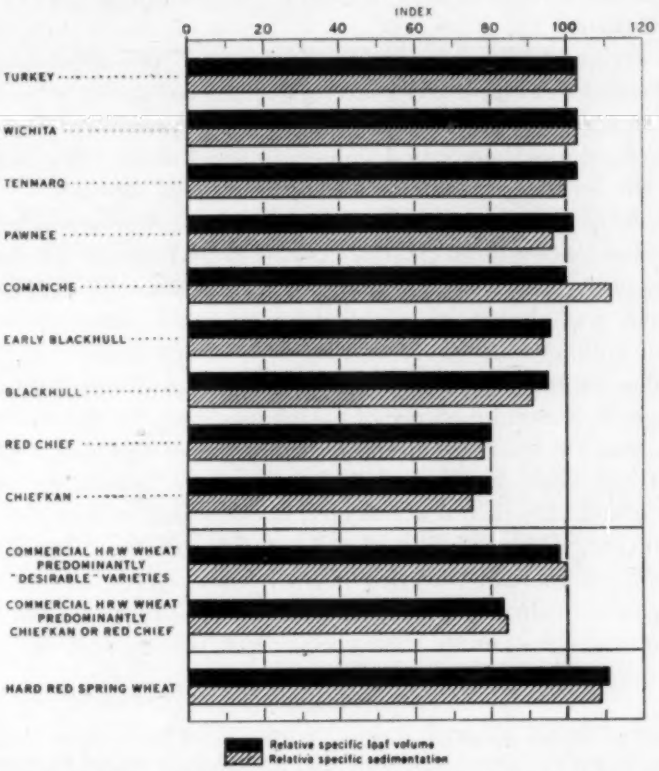

Fig. 4. Relative gluten quality of different varieties of hard red winter wheat, commercial lots of hard red winter wheat, and variety samples of hard red spring wheat as measured by specific loaf volume and specific sedimentation.

tation values of 3.0 or higher, and of the 21 samples of this group that had specific sedimentation values of less than 3.0, all but seven were shown by the specific loaf volume data to have gluten of less than average quality. From the data obtained, therefore, it seems proper to conclude that for experimentally milled hard wheat flour from the 1946 crop, it should be possible to segregate with a reasonable degree

[3] The data in respect to the comparative bread-baking quality and gluten quality of different varieties and classes of wheat presented in this paper are intended only for the purpose of comparing different methods of testing. The data are grossly inadequate in quantity to be used in any attempt to establish the relative quality of different varieties or classes of wheat and should, therefore, not be considered from that standpoint.

of accuracy flour from the varieties Chiefkan and Red Chief along with any other flour of markedly inferior gluten quality by means of the specific sedimentation value.

Summary

A quick sedimentation test of extreme simplicity, in which the rate of sedimentation of the solid phase of an acidulated suspension of flour in water is measured, has been devised for estimating the bread-baking quality of flour.

In a series of 135 experimentally milled flours representing individual varieties of hard red winter and hard red spring wheat grown at different stations and commercial hard red winter wheat from different markets, the sedimentation value was found to be fully as good an index of the bread loaf volume as was the protein content.

Flour from Chiefkan and Red Chief wheat (varieties of generally recognized inferior gluten quality) tends to be properly evaluated in respect to potential bread loaf volume by the sedimentation test, while the protein test almost invariably overestimates (often greatly) the bread loaf volume that can be attained from such flour.

Specific sedimentation (sedimentation value divided by protein percentage) is a useful measure of gluten quality. In the series of 135 flour samples, the inferior gluten quality of the 23 samples of Chiefkan or Red Chief flour (or flour milled from wheat consisting predominantly of those varieties) was reflected in every instance by the specific sedimentation. The relative gluten qualities of flour from nine of the leading commercial varieties of hard red winter wheat were, with one exception, evaluated in essentially the same order by their specific sedimentation values as by their specific loaf volume values.

Acknowledgments

The author wishes to acknowledge the assistance of L. P. Reitz, Agronomist, Agricultural Experiment Station, Lincoln, Nebraska, who furnished the pure variety samples of hard red winter wheat; of Tyler Hartsing, Grain Technologist, Grain Branch, Production and Marketing Administration, who milled the flours; of C. C. Fifield, Baking Technologist, Bureau of Plant Industry, Soils, and Agricultural Engineering, under whose direction the baking tests were made and who assisted in the statistical analysis of the data; and of Mary Stutzman, Scientific Aide, Grain Branch, Production and Marketing Administration, who performed the protein tests.

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PROPORTION OF HULL IN SOME NORTH DAKOTA BARLEY VARIETIES, AS DETERMINED BY THE AIR JET TECHNIQUE

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(Received for publication June 5, 1947)

The hull component of the barley kernel is of little use for feeding purposes. It is, nevertheless, a substantial portion of the threshed grain, constituting as much as 10% or more of the whole kernel. A knowledge of the hull percentages in various barley varieties is important to the breeder who wishes to develop varieties relatively low in hull content. Information regarding hull percentages has been difficult to secure because of the labor and time required to determine hull values by manual methods.

Fraser (1944) described an air jet technique for dehulling seeds which appeared to supply the answer to the problem of obtaining reliable information regarding hull percentage by a fairly rapid, simple method. Essentially the method consisted of violently agitating the soaked or tempered grain by an air jet while enclosed in a metal cup. The cup was $2\frac{1}{4}$ inches in both length and diameter and was fitted for barley dehulling with a wire screen which lined the bottom, sides, and top of the cup. The top screen was removable. The jet was operated under a pressure of approximately 20 pounds per square inch. This caused the kernels vigorously to bombard the screen-covered confining walls of the cup, while the hulls were forced through the top screen, leaving the hulled kernels in the cup. Ten to 15 minutes sufficed to attain satisfactory removal of the hulls. The dehulled kernels were air-dried to remove moisture absorbed during the tempering period,

then weighed to ascertain the loss in weight due to dehulling. A 3-g charge was found to be quite satisfactory. This method gave very slightly lower hull percentages than the hand method, due to less complete removal of the hull from the crease of the kernel. Hannchen was especially difficult to dehull.

In view of the interesting data obtained by use of the air jet method in dehulling Western Canada barley, it was decided to construct a similar apparatus and employ it in ascertaining the hull content and ease of hull removal in several varieties of barley grown on experimental plots at various locations in North Dakota.

Materials, Equipment, and Methods

Materials. The material used for the principal part of the investigation consisted of five varieties of barley grown at six stations in the state. The varieties and stations are identified in Table I. These barleys were grown under comparable conditions, using farm practices common in the area. Each sample was passed several times through

TABLE I

AVERAGE VALUES OF HULL REMOVED BY ALL TESTS

Length of run	Varietal averages				
	Tregal	Kindred	Manchuria	Trebi	Plush
Minutes	%	%	%	%	%
5	8.5	7.7	7.4	6.5	6.6
10	11.0	10.7	9.8	8.4	8.5
15	11.7	11.2	11.6	10.1	10.1
20	12.8	12.6	11.8	10.9	11.0
Mean	11.0	10.5	10.1	9.0	9.0

Significant difference		
	5% level	1.10
	1% level	1.46

Length of run	Station averages					
	Langdon	Williston	Edgeley	Minot	Dickinson	Fargo
Minutes	%	%	%	%	%	%
5	7.1	8.1	7.1	7.7	7.7	6.3
10	10.5	10.3	10.0	9.8	8.9	8.6
15	11.9	11.3	11.4	11.1	10.2	9.7
20	12.5	11.2	12.2	11.4	11.6	12.1
Mean	10.5	10.2	10.2	10.0	9.6	9.2

Significant difference		
	5% level	1.06
	1% level	1.34

an Emerson dockage tester to remove extraneous material. In certain of the samples, barley kernels persisted in tailing over because of extremely long awns. These kernels were finally passed through a scouring machine and the long awns removed in this manner and then the kernels once more put through the dockage separator. The cleaned samples were used for test weight, moisture, protein, and hull determinations.

Equipment. The apparatus used for dehulling was essentially that used by Fraser and had approximately the same dimensions (Figure 1).

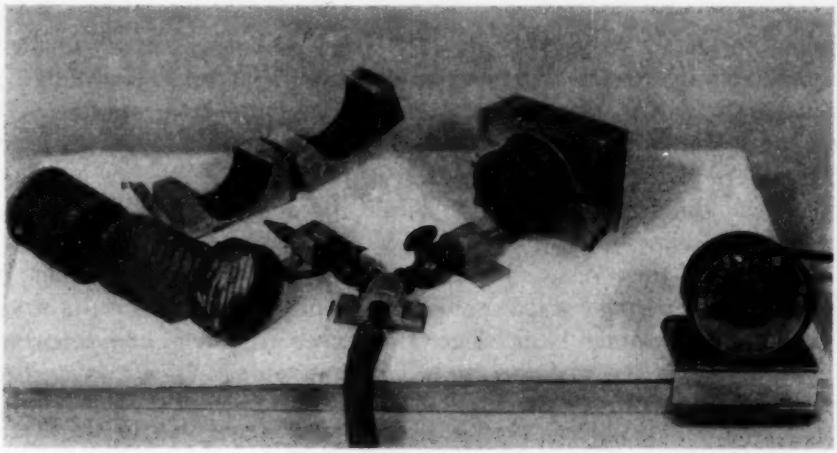

Fig. 1. Apparatus used for dehulling barley kernels. The dissassembled unit is shown at left, with container, wire cage, and cover, while the huller ready for use is at the right.

A metal cylinder closed at one end was fitted with a wire cage of slightly smaller size. The cage was constructed from No. 15 tempered steel wire flax screen. A cap was also made from this wire to close the container and prevent loss of kernels while permitting free egress to hulls. The container was held quite firmly in a hollowed-out wooden block hinged at the side and fastened during operation by a spring which hooked over a suitable pin. This permitted easy and rapid fastening and removal of the container. The inner portion of the block was lined with felt to assist in holding the container firmly during the dehulling operation.

As shown in Figure 1, two containers were used simultaneously with two brass air jets, 1.2 mm inside diameter. These branched from a central tube in the form of a "Y." The position of the air jets was quite critical because it affected the uniformity of dehulling. A compressor driven by an electric motor supplied compressed air at a line pressure of 17.5 pounds per square inch. Duration of dehulling

was controlled by an automatic timing device which is shown at lower right. It was found necessary to place the air jet nozzle carefully just inside the cover screen at the lower edge with the container lying on the side, as in the complete assembly pictured on the right of the figure. When the compressor motor was started, the kernels were violently agitated and thrown against the lining.

Method. Preliminary trials with barley tempered for various periods and temperatures failed to give entirely satisfactory results. It was difficult to secure satisfactory removal of the hull, and the hull values were low. This may have been due in part to absorption of water by the kernel during tempering, although a few samples of dehulled barley left exposed in the laboratory overnight did not change appreciably in weight. A few experiments with Aerosol (1% solution) failed to improve the results obtained by preliminary tempering. It was at first thought that the unsatisfactory results might be due to incomplete penetration of the water, and that this reagent would improve the ability of the water to soften the hull. Various tempering periods and temperatures were tried without appreciable success.

In view of these results it was decided to see what would happen if no initial tempering at all was used. Surprisingly good results were immediately obtained, which agreed closely with the values reported by Fraser, and there appeared to be little cause for further study of the effect of tempering. The temperature and humidity of the laboratory were fairly constant, averaging 80°F and 32% respectively. These factors, particularly relative humidity, should be taken into consideration when employing the air jet method.

Using the method without tempering, a moisture loss of 0.1 to 0.2% was occasioned by dehulling for 15 minutes, the larger loss occurring with barley containing 11.8% moisture. These changes are, of course, insignificant and accordingly the kernels could be weighed immediately after dehulling without the necessity of correcting for loss or gain in moisture content. Several determinations were made at higher relative humidities (58–59%) to ascertain their effect on hull removal. The samples were exposed to this humidity for one and one-half hours previous to dehulling. Hull removal was incomplete under these conditions. Apparently the relative humidity of the laboratory has a marked effect on the facility with which the hulls may be removed from barley.

The procedure finally adopted consisted of placing a 3-g charge of clean barley in the apparatus and starting the blast. Various lengths of time were used for dehulling to secure information on the relative rate of hull removal from various barley varieties. While one pair of samples was being dehulled, the next pair was weighed out. The

provision of two air jets and two barley dehullers of course doubled the number of samples handled in a specified time. After dehulling, the kernels were removed from the container, any loose hulls separated, and the kernels weighed to the nearest centigram. If the difference between duplicates exceeded 6 cg, the determination was repeated. No hand peeling determinations were made in view of the comparative data from the air jet and hand methods reported by Fraser.

Results

Some of Fraser's data are reproduced below and compared with results secured by the present method:

	Wt. of hulled barley, g
University of Saskatchewan:	
Knife peeling	2.64
Air jet	2.67
North Dakota Agricultural Experiment Station:	
Air jet, 10 min.	2.74
Air jet, 15 min.	2.69
Air jet, 20 min.	2.67

The values are not strictly comparable because the varieties are not identical in the two sets of barley samples, and in addition they were grown under different environments. However, the results indicate very good agreement between the methods, particularly with the longer dehulling periods in the North Dakota modification. The hand method tended to give higher values because of more complete removal of hull from the crease, but the differences from the air jet method are very small and doubtless would not affect the comparative ranking of the varieties by the two procedures. The appearance of a representative sample of barley as dehulling proceeds is shown in Figure 2.

Averages for Varieties and Stations. The average percentage of hull removed from the varieties shows that there are substantial differences in this component among the five barley varieties (Table I). The proportion of hull removed from each barley with increased length of treatment rose rather consistently for each variety with the greatest effect evident at the 20-minute period. The percent of hull removed with varying length of dehulling time is shown graphically in Figure 3. Tregal and Kindred show a distinct change in rate of hull removal after 10 minutes and Manchuria after 15 minutes, while the relation between hull removal and time is almost linear up to 15 minutes for Plush and Trebi. However, the lines bend at the 15-minute point, demonstrating a curvilinear effect. There is some indication of a slight change in the latter two curves after 15 minutes. The loss after

15 minutes may include some abrasion of the kernel itself although none was evident under slight magnification (Figure 2). The change in hull content with treatment is clearly shown in the photograph, with evidence of rather incomplete hull removal after 10 minutes. Characteristic differences in hull content are evident in Figure 3 and support the belief that barley varieties differ inherently in this component. Further conclusive evidence on this point is presented in the analysis of the data given in the following section. Tregal is quite similar to Kindred, and Trebi to Plush, in the proportion of hull, but there are very significant differences in hull content between the first and the

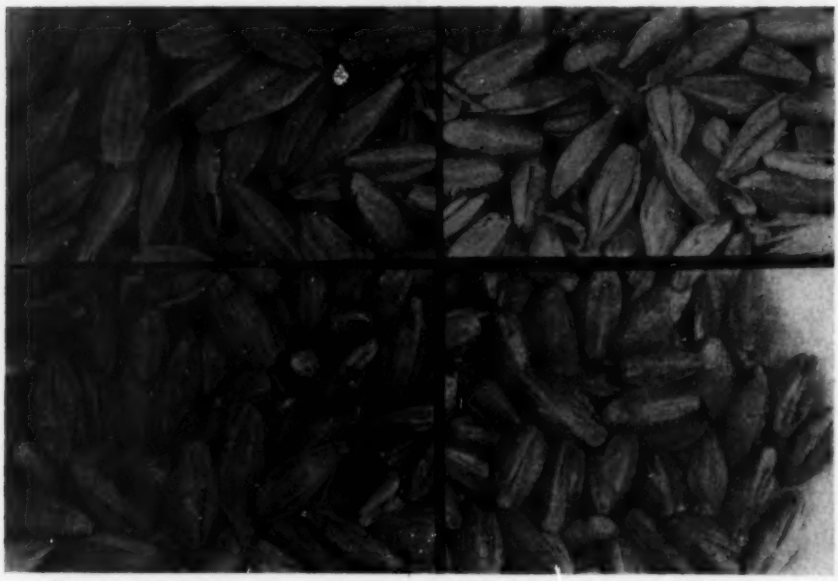

Fig. 2. Appearance of the barley kernels following different dehulling periods. Upper left, original sample before dehulling. Upper right, after 5 minutes' dehulling. Lower left, after 10 minutes' dehulling. Lower right, after 15 minutes' dehulling.

latter pairs. Manchuria is significantly higher than Trebi and Plush and lower than the others. The differences required for significance at the 5 and 1% levels are shown below the varietal averages. These were calculated from the analysis of variance shown in Table II.

Kernel size is very markedly affected by variety, and accordingly hull thickness may not vary directly with the percentage of hull. In the present study, for example, when the weight of hull per kernel for Manchuria and Trebi was compared including data for all six stations, the latter variety was found to have a hull weight of 0.0041 g per kernel, while the former had a corresponding value of only 0.0036 g. Trebi has larger kernels than Manchuria and this factor more

than offsets the higher percentage of hull when each kernel is considered separately. Barley experts generally recognize that Trebi has a thicker hull than Manchuria.

Averages for the six stations are also presented in Table I. It is apparent that the station where the barley was grown had less effect on the proportion of hull than the variety. This is the chief point of

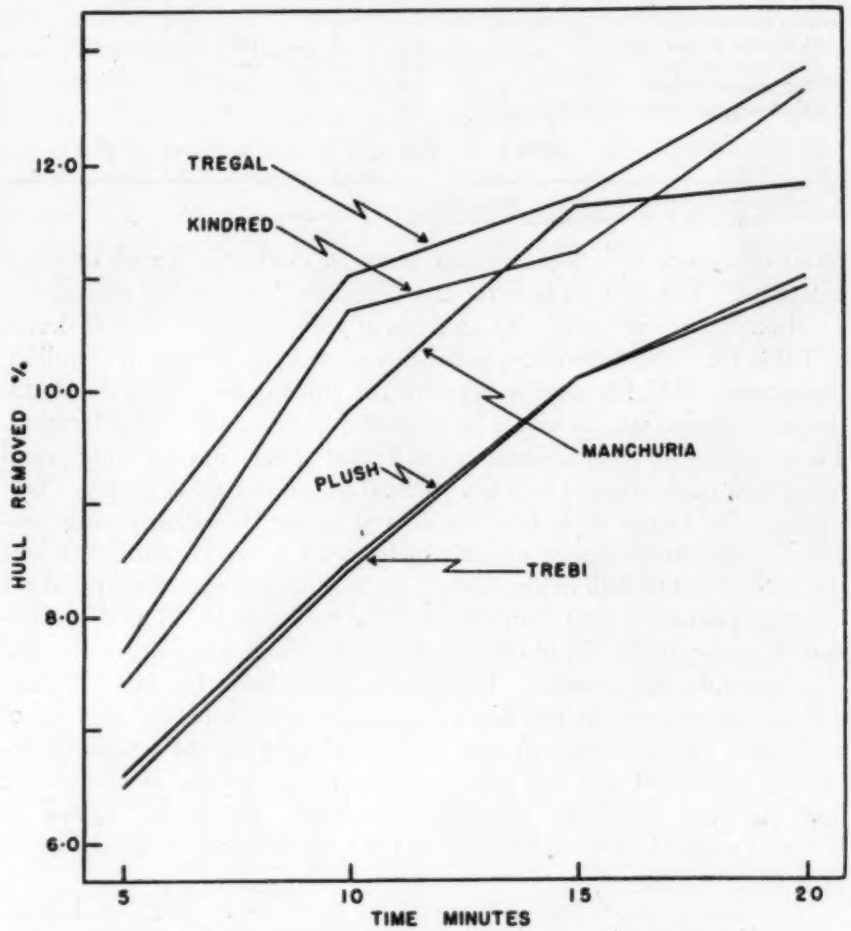

Fig. 3. Relations between percentage of hull removed and dehulling time for five varieties of barley.

interest in the station data since these in general resembled the results from the varieties. The range among stations is only 1.3%, which is approximately the 1% level of significance, while for the varieties it is 2.0%. Little can be stated regarding the effect of the environment in detail on hull percentage, since the environments during seasonal growth at Dickinson and Fargo differ markedly, yet there is no signi-

TABLE II
ANALYSIS OF VARIANCE OF BARLEY HULL PERCENTAGES

Source of variation	Degrees of freedom	Variance	F
Between varieties	4	19.49	25.64**
Between stations	5	4.52	5.95**
Between times	3	114.11	150.14**
Interactions:			
(Varieties×stations)	20	1.93	2.53**
(Varieties×times)	12	0.60	
(Stations×times)	15	1.67	2.20*
(Varieties×stations×times)	60	0.76	
Total	119		

* Denotes 5% level of significance was attained.
** Denotes 1% level of significance was attained.

ficant difference in hull percentage between the barley grown at these locations. The same is true for the Williston and Edgeley stations.

Analysis of Variance. An analysis of variance of the data is shown in Table II. The differences between varieties, stations, and dehulling times were all highly significant with the third source being decidedly the most important, as would be expected from *a priori* consideration. The analysis confirms the findings of Fraser (1944) and the widespread belief that percentage of hull is a varietal characteristic of barley. Obviously this factor should be considered in any barley-breeding program. The environment under which barley is grown also influences the proportion of hull in the kernel; large plump kernels would have a lower proportion of hull than thin or shrivelled kernels. The size of the kernel in any barley is, of course, determined in a large degree by the conditions during growth. The interactions show that the varieties did not all respond in the same manner in respect to the amount of hull when the environment was varied. Changing the length of dehulling treatment did not cause significant variations in amount of hull removed from the varieties. However, the barley grown at different stations did not respond in the same manner to variations in time of dehulling.

Correlation Coefficients. Correlation coefficients calculated from the dehulling data for different lengths of time are shown below:

Variables correlated (figures represent duration of test)	r_{xy}
5′ and 10′	0.665**
5′ and 15′	0.524**
5′ and 20′	0.178
10′ and 15′	0.765**
10′ and 20′	0.471*
15′ and 20′	0.454*

Note: All correlation coefficients are positive.
* Denotes significance at 5% point.
** Denotes significance at 1% point.

The value of the coefficient between the five-minute and the other three determinations increases as the time decreases, but the highest value, +0.665, is still too low to permit a precise prediction of hull percent from the five-minute determinations. Nor can results secured from 10 minutes of dehulling be used to predict those attained from 15 minutes. The reasons underlying this lack of agreement between different times of dehulling are not clear at the present time. They may lie in the varying facility with which the hull is removed from different samples of barley. There is, no doubt, some slight abrasion of the kernel itself, although, as pointed out previously, there was little visual evidence of this under magnification (Figure 2). There may also be slight variations in the proportion of the hull remaining in the crease among the various samples. The five-minute run obviously was too short, resulting in incomplete removal of the hull, while the 20-minute determinations were the most apt to result in kernel abrasion due to the lengthy time of exposure of the kernel to bombardment. In addition the latter period was tedious and required too much time. This appears to narrow the choice to the 10- or 15-minute periods for the determination of hull percentage.

The 15-minute period is the best to use because it gives good hull removal with not too much exposure of the kernel to damage. The 10-minute procedure results, however, in fairly satisfactory dehulling and very little opportunity for kernel loss to occur. It is also more economical of time since there is opportunity for weighing out the original samples and reweighing the dehulled samples while the dehulling is being done. These weighings, coupled with recording the data and removing unsuitable material from the original sample and stray unattached hulls from the dehulled barley, rather completely occupy the operator.

The relative rates at which the five varieties relinquished their hulls are shown in Figure 4. These rates, or hull removed per minute, were computed by calculating the ratio H/T, where H = percentage of hull removed and T is time in minutes. It is evident that the rate decreased as the time of dehulling increased. Varietal differences in the rate were most evident at 5 and 10 minutes. It is possible that if other barley varieties were studied, these differences would be more marked. An analysis of variance of the dehulling rates for the four times indicated that significant differences between varieties existed only for the five-minute treatments. The analysis is shown in Table III. This is the time at which the rate of hull removal is most rapid and accordingly the point at which differences in the degree of tightness with which the hull clings to the kernel should be most apparent. As dehulling progresses the rate decreases, as shown in Figure 4.

TABLE III

ANALYSIS OF VARIANCE OF DEHULLING RATE AT FIVE MINUTES

Source of variation	Degrees of freedom	Variance	F
Between varieties	4	0.16	3.26*
Between stations	5	0.09	1.78
Interaction: Varieties×stations	20	0.05	
Total	29		

* Denotes 5% level of significance was attained.

This would be expected because, as the proportion of hull is progressively removed, the remainder subject to separation will become steadily less, and the rate will decrease.

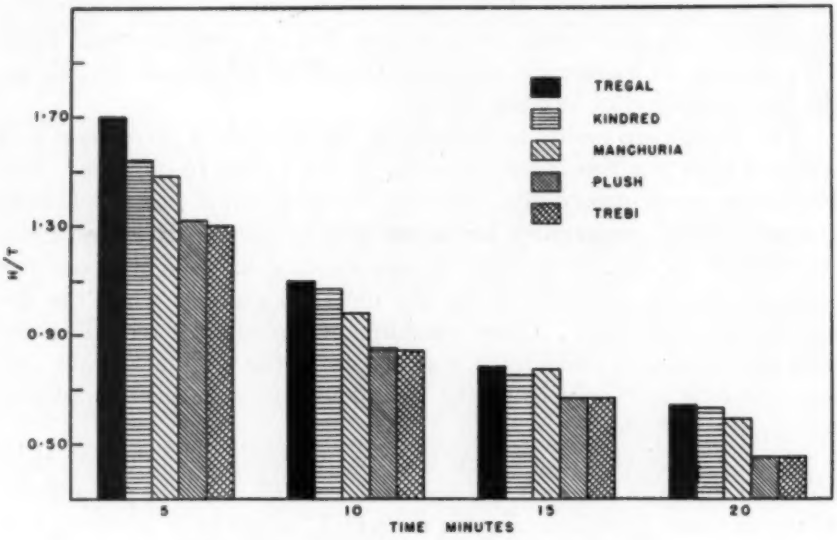

Fig. 4. Comparative rates per minute of hull removal at different lengths of time for five varieties of barley.

Conclusions

Barley can be readily hulled by the air jet method with a satisfactory degree of reproducibility. No tempering preliminary to hulling is required if the relative humidity is not above 35%, at least for barley grown in North Dakota. This considerably simplifies the procedure. Since the variety-time interaction is nonsignificant, it appears that any time of hulling between 5 and 20 minutes, inclusive, to differentiate varieties in respect to proportion of hull might be

employed. However, if fairly precise hull percentages are required, 15-minute determinations should be employed since the correlation coefficient between any pair of times is too low to permit the use of a prediction equation. If the degree of tightness with which the hull is held is to be ascertained, the five-minute period should be employed. No doubt other seeds can be hulled satisfactorily in this apparatus by employing suitable modifications, as suggested by Fraser.

Summary

A modification of the air jet method developed by Fraser for determining the proportion of hull in North Dakota barley is described. This method consists of propelling the barley violently against a wire cage lining a metal container. An air jet is employed to propel the barley. Various lengths of treatment were studied and a period of 15 minutes appeared most satisfactory. No preliminary tempering was found necessary.

Very significant differences in hull percentage were found among barley varieties, while location of growth had less effect than variety. Length of hulling treatment had, of course, a very large influence on proportion of hull removed. A significant relation was noted between variety and rate of hull removal only when a five-minute dehulling period was employed. Correlation coefficients between hull removed by different treatments were too low to justify prediction of hull removed by one method from information secured by any of the other three treatments.

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EFFECT OF PHOSPHATES ON SODIUM CHLORIDE DURING THE ASHING OF SALTED CEREAL PRODUCTS

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(Received for publication March 31, 1947)

An estimation of the original flour ash in flour mixes, breads, and other salted cereal products is frequently needed. Although the official A.O.A.C. method for ashing cereal products, as well as that of Bailey (1937), gives accurate and reproducible results on the original flours, neither of these methods gives reliable results for the original

flour ash in salted products. A number of special methods for this purpose have been proposed but are not entirely satisfactory in that they have all been based on the supposition that sublimation of sodium chloride during incineration is the only mechanism by which loss of this salt occurs.

The purpose of the present study was to disclose, if possible, transformations and reactions of sodium chloride other than direct sublimation during the ashing procedure and to determine which practical conditions of temperature and duration of ashing can be used to give more satisfactory results.

Kalning (1920) proposed a method for determining the original flour ash in bread by the difference between the total ash and the sodium chloride content. His method consists of extraction of the charred bread with acidulated hot water and estimation of sodium chloride in this solution by titration of the chlorides: total ash is determined in a separate operation. The use of finely powdered calcium carbonate to prevent volatilization of sodium chloride during ashing was proposed by Zunino (1934) to permit titration of sodium chloride in the ash. Sgarzi (1934) modified the extraction of sodium chloride by using alkaline solution and recommended the addition of methanol before ashing. A useful but complicated method was introduced by Knottnerus (1916) in which the sodium chloride is extracted with acidulated water and the ash determined on the extracted bread. The sodium chloride is titrated in an aliquot of the extract so that the total sodium chloride in the remainder of the solution can be precipitated by the addition of an exactly equivalent quantity of standard silver nitrate solution. The precipitate is washed and the filtrate plus wash water (containing any sodium in the form of sodium nitrate) is evaporated and the residue incinerated and weighed. The sum of this value and the ash value of the extracted bread minus the sodium oxide equivalent to the total quantity of sodium chloride determined by titration represents the ash content of the original flour.

Comparison of these methods has shown that the results for sodium chloride-free ash are lower than the original flour ash, except in the method of Knottnerus. In addition, the sodium chloride values when determined in the ash are not equivalent to the surplus of the ashes, and the sodium chloride-free ash thus calculated is always higher than expected. This observation suggests that some reaction of sodium chloride beyond its simple sublimation must occur during the ashing procedure. The possibility that sodium chloride may be partially converted to sodium carbonate during ashing was suggested by Pelshenke (1938). The occurrence of sodium carbonate should be detectable by an increase in ash alkalinity. The alkalinity of the ash is

a very erratic value, however, and is not closely correlated to the sodium content of the ash. Moreover, its measurement is difficult and the results obtained are greatly influenced by the method used. It seemed desirable to find some other means of disclosing the reactions of sodium chloride during ashing.

Although the Knottnerus method is capable of giving accurate results, it is cumbersome and time-consuming to an extent unsuitable for extensive series of analyses. This method sidesteps the pitfalls of ashing bread in the presence of sodium chloride but does not explain the necessity for such a procedure. Since it was our purpose to clarify this problem, we used the direct ashing procedure.

Experimental

The first experiments were designed to measure the amount of sodium chloride that is volatilized and the amount, if any, that is converted to other compounds during ashing. For this purpose, two samples of bread with different salt contents were used. The bread was sliced, dried, ground, and mixed thoroughly. For ashing, 3.0-g. samples were weighed into quartz crucibles. In order to hasten the process, incineration was interrupted three or four times to add a few drops of distilled water. The temperature did not exceed 500°C. Approximately 5 hours were required to complete the incineration and obtain satisfactory light gray ashes.

The sodium chloride was determined in the ash and also in the water extract of the bread samples. For the latter, 5.0-g. samples were washed with three successive 50-ml. portions of boiling distilled water. The extract was cooled and diluted to 250 ml. A 150-ml. aliquot was used for chloride titration according to Volhard. In a similar manner, the ash was extracted with hot water acidulated with a few drops of nitric acid and the chloride titrated as above.

The results obtained for sodium chloride in the bread extract, the ash extract, and the unextracted bread ash as well as the original flour ash are presented in Table I.

It is clear from these data that significantly less sodium chloride was found in the ash extract than in the bread extract. A difference of this amount would hardly be expected to be due to sublimation since the ashing temperature did not exceed 500°C. These data also show that the original flour ash is not reliably indicated by the difference in bread ash and the sodium chloride titrated in the ash extract. The ingredients of bread other than flour and salt influence this relationship, and it seems possible that incompleteness of extraction or the presence of a compound which is not determinable by chloride titration may be involved. The latter is also suggested by the increase

TABLE I

VOLATILIZATION OF SODIUM CHLORIDE UPON ASHING BREAD
(Results based on 3.0 g. bread)

Bread ash	Flour ash	NaCl in bread extract	NaCl in ash extract	Calc. loss of NaCl in ashing	Bread ash minus NaCl in bread extract	Bread ash minus NaCl in ash extract
mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
158.7	56.4	117.9	85.9	32.0	40.8	72.8
96.4	56.4	54.3	28.1	26.2	42.1	68.3
121.3	68.7	71.7	29.6	42.1	49.6	91.7
122.5	68.7	71.7	31.8	39.9	49.8	90.7

in the loss of sodium chloride upon ashing with the increase in original flour ash.

Pelshenke (1938) postulated that a reaction of organic compounds with sodium chloride occurs during the ashing process. When pure sucrose was dissolved in salt water and ashed, no evidence of sodium chloride loss was observed. Materials containing metallic impurities, however, caused appreciable loss of sodium chloride upon ashing. Phosphates in particular were observed to be effective in this reaction and were consequently specially studied in view of the phosphates normally used in flour mixes and dough improvers, as well as those contained in the flour itself.

A solution was prepared which contained 0.6% potassium dihydrogen phosphate and 0.25% monocalcium phosphate. When 5 ml. of this solution was evaporated and incinerated several minutes, a residue of 39.6 mg. was obtained. Known amounts of sodium chloride were mixed into the phosphate solution and incinerated 3 minutes in an open Bunsen flame. The residue was cooled and weighed. This process was repeated two or three times, using one minute heating, until constant weight was obtained. The residue was taken up in 5% nitric acid solution and sodium chloride determined by titration as described above. The results of these experiments are presented below:

NaCl in solution	*NaCl in residue*	*Total residue*	*NaCl-free residue*	*Blank*	*Loss of NaCl*
mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
60.0	26.3	82.4	56.1	39.6	33.7
60.0	29.1	84.6	55.5	39.6	30.9

These data show clearly that about 50% of the sodium chloride had reacted with the phosphates under these conditions and was no longer titrated as the chloride.

Further experiments were conducted using potassium acid phosphate alone. This compound, as is well known, loses one mole of water when heated and fuses as the metaphosphate (KPO_3). A 0.1 *N* solution of potassium dihydrogen phosphate was prepared and found to yield blanks in good agreement with the theoretical values. Aliquots of this solution to which known quantities of sodium chloride were added were treated as described above. The results obtained are given in Table II.

TABLE II

ASH RESULTS OBTAINED WITH 0.1 *N* POTASSIUM DIHYDROGEN PHOSPHATE CONTAINING KNOWN AMOUNTS OF SODIUM CHLORIDE

Total residue	NaCl in solution	NaCl in residue	NaCl decomposed in terms of		Residue free of sodium oxide and chloride	Blank (KPO_3)
			NaCl	Na_2O		
mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
64.6	14.3	0	14.3	7.6	57.0	57.4
135.7	28.6	0	28.6	15.2	120.5	119.0
78.2	28.6	7.9	20.7	11.0	59.3	59.8
79.2	28.6	5.6	23.0	12.2	61.4	60.4
77.1	28.6	2.2	26.4	14.0	60.9	60.4
100.2	57.3	22.8	34.5	18.3	59.1	59.8
101.4	57.3	25.6	31.6	16.8	59.0	59.8
159.4	58.4	14.7	43.7	23.2	121.5	119.0
206.8	116.8	55.5	61.3	32.5	118.8	119.0

These data show that the decrease of chloride in the residue is correlated with the sodium chloride–phosphate ratio. If an excess of phosphate is present during incineration, no titratable chloride can be found in the residue, and apparently under these conditions the chlorine had been quantitatively liberated from the sodium chloride. Losses of sodium chloride were considered here to be the result of a reaction with the phosphate whose end products were sodium oxide and hydrogen chloride. When the amounts of sodium oxide are calculated on this basis, it is evident from the data in Table II that the reaction involved is of this nature, since the calculated values for the sodium chloride–sodium oxide free residues are in close agreement with the blanks.

A study was made of the influence of the sodium chloride–phosphate ratio, as well as of the temperature and duration of incineration, upon the sublimation losses. In the foregoing tests, a dull red temperature was generally reached during the incineration. To increase the amounts of sodium chloride sublimed for this purpose, temperatures of 600°C and higher were used. The data obtained were tabulated as follows:

Period of incineration, minutes	*Decrease in weight* 1 mg.	2 mg.	3 mg.
1.5	30.2	43.2	39.9
1.5	1.5	1.8	3.9
5	0.4	0.8	3.7
5	0.0	0.7	2.7
5	0.0	0.8	2.4
NaCl titr. in residue	0	11.1	55.5
NaCl titr. in solution	29.2	58.4	116.8
NaCl : Phosphate ratio	1/2	1/1	2/1

It is apparent from these data that the largest loss of weight occurs at the start of the heating treatment, and becomes a fairly constant value after a 3-minute incineration period. This is followed by a slow constant loss due to sublimation. It thus appears that disintegration occurs in the first period of heating, when chlorine alone volatilizes, while the intact portion of sodium chloride is subject to sublimation later. No sublimation occurred when the phosphate was in excess, since the initial reaction consumed it entirely with liberation of chlorine. When the sodium chloride is in excess, however, considerable sublimation is to be expected. Practically, sublimation can be avoided only when the sodium chloride–phosphate ratio is held under 1. Thus, it is essential when ashing bread and other salted cereal products in which salt is in excess of this ratio to use as low a temperature as possible and to reduce the duration of incineration by moistening the ash at frequent intervals with a few drops of distilled water or ethanol.

A series of low grade and patent flour samples and a sample of bran to which known amounts of sodium chloride were added were ashed under these conditions to check the reliability of this procedure. The results obtained are given in Table III.

TABLE III

ASH RESULTS WITH FLOURS CONTAINING VARIABLE AMOUNTS OF SODIUM CHLORIDE

(Results based on 3.0 g. dry matter)

Material	Flour ash	NaCl added	Total ash	NaCl in ash	Na_2O corresp.	Na_2O, NaCl free ash
	mg.	mg.	mg.	mg.	mg.	mg.
Low grade flour	48.7	57.2	92.5	29.2	14.9	48.4
Low grade flour	49.0	60.0	95.8	31.6	15.1	49.1
Low grade flour	48.1	60.0	96.4	33.9	13.8	48.7
Low grade flour	48.5	60.0	96.8	34.3	13.6	48.9
Low grade flour	47.0	29.9	62.3	0.5	15.6	46.2
Patent flour	19.1	60.0	73.0	46.2	7.5	19.3
Patent flour	18.0	60.0	68.6	42.3	9.2	17.1
Patent flour	18.8	60.0	66.4	36.2	12.6	17.6
Bran	147.8	60.0	187.2	15.8	23.4	148.0

The calculated sodium oxide and sodium chloride-free ash values are in close agreement with the original ash values and thus show that this procedure can be relied upon for estimation of the original flour ash in salted cereal products where salt is the only ash-bearing ingredient added to the flour.

Discussion

In view of the effects of phosphates on sodium chloride during the ashing of cereal products, it is apparent that the theories which have been advanced in the past attributing the losses of sodium chloride during ashing wholly to sublimation need modification. Under the usual conditions of ashing, it is suggested that a reaction occurs between the sodium chloride and the phosphates with chlorine being liberated and volatilized and sodium oxide being one of the end products. The reactions may be generalized as follows:

$$2\ KH_2PO_4 + 2\ NaCl \longrightarrow 2\ KNaHPO_4 + HCl$$

$$2\ KNaHPO_4 \longrightarrow K_2Na_2P_2O_7 + H_2O$$

$$K_2Na_2P_2O_7 \longrightarrow 2\ KPO_3 + Na_2O$$

From a practical viewpoint, it is irrelevant whether or not the sodium takes part in the formation of a pyrophosphate molecule, provided the end product is sodium oxide and the calculations are made on the sodium oxide basis. It is probable that the actual reaction is more complicated than the series outlined above and must await further investigation to be fully clarified.

Summary

The estimation of original flour ash in salted cereal products is unreliable when the ashing is done by means of the common ashing methods.

In the presence of acid phosphates, sodium chloride reacts during incineration, allowing the chlorine to volatilize while the sodium remains in the ash, apparently as the oxide. When phosphates are in sufficient excess, this reaction is very rapid and almost quantitative. Sublimation of sodium chloride only occurs when the sodium chloride–phosphate ratio exceeds 1 at temperatures above 600°C.

The direct ashing method is suitable for estimation of original flour ash in salted cereal products if care is taken to incinerate at temperatures not higher than 500°C and if the phosphate effect is taken into account by computing as sodium oxide the sodium chloride lost during incineration and subtracting from the sodium chloride-free ash value.

Acknowledgment

The authors wish to express their sincere thanks to H. Obermeyer, Research Department, General Mills, Inc., Minneapolis, for editing the English translation of the original manuscript.

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STUDIES ON BREAD STALING. II. WATER RELATIONSHIPS DURING STALING OF BREAD CRUMB AND THE RETROGRADATION OF STARCH[1,2,3]

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(Received for publication August 15, 1947)

It is commonly observed that, during staling, bread loses its plastic properties, becomes tough, and the crumb loses its ability to swell when added to water (Balland, 1892). It was early shown that this change will occur without any loss of water (Boussingault, 1852). Conversely, it has been shown that if the fresh bread is dried quickly to a low moisture content, the structural evidences of staling fail to develop or do so at a much slower rate (Katz, 1928, 1934; Katz and Weidinger, 1934). These facts indicate that some change in structure is developed in bread during staling which involves or is aided by the water present in the bread. Water of fresh bread acts as a plasticizer, that is, serves as a fluid medium in which motion of the solid particles past one another can take place. It could be that during staling the water becomes

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[1] Paper No. 2355, Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] The subject matter of this paper has been undertaken in cooperation with the Committee on Food Research of the Quartermaster Food and Container Institute for the Armed Forces. The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsements of the War Department.

[3] The first paper in this series entitled "Studies on Bread Staling. I. Role of Starch" by T. J. Schoch and Dexter French appeared in Cereal Chem. 24: 231–249, 1947.

bound to the solid phase and thereby loses its ability to act as a plasticizing medium in which relative movement of the nonwater particles can occur. If this were true, it should be reflected in the strength and extent of water binding by the solid. If, however, no change in the water binding occurs during staling, it would have to be concluded that the structure which develops must involve the formation of cross linkages between the nonwater elements of the bread. The part played by water in this case would be limited to its function as a medium in which rearrangement of the nonwater components could take place, i.e., in which they could move kinetically to positions relative to each other such as would be required before cross bonding could occur between them.

If, during the course of staling, the water present in the fresh bread should become combined with the nonwater components, it is conceivable that such binding could be either chemical or physical in nature. If chemical binding should occur, such water would no longer exert a vapor pressure and such a process would be reflected in an increase in the dry weight of the bread substance. That no chemical binding of water does occur during staling was indicated experimentally by the fact that the dry weight (after one hour in a forced draft oven at 130°C) of a sample of fresh bread was the same as that of an identical sample of the same bread after it had been allowed to stale while stored in an airtight container for a period of a week at 6°C.

Physical binding of water by nonaqueous materials results only in a decrease in the activity or relative vapor pressure of the water bound. The strength of binding (degree of decrease in activity resulting from the binding) and the extent of binding (amount of water which has been reduced to any chosen activity) may best be estimated in terms of the relative vapor pressure–water content curve per unit weight of the nonaqueous substance under consideration (Briggs, 1932). If the physical binding of the water in bread should change during the staling process, this change would be reflected in the relative vapor pressure–water content curves made on fresh and stale samples of the bread crumb. Should these curves prove to be identical for fresh and for stale crumb, it would be obvious that no change in the water-binding capacity of the nonaqueous components of the bread had occurred, and that the increase in structure must have resulted from cross linkages between the solid elements. If, on the other hand, the relative vapor pressure–water content curve for the stale bread components indicated a large increase in the amount of water bound over that of the fresh bread components, it could be interpreted that the structure developed was due merely to the increased resistance to shear resulting from the decrease in the amount of plasticizer (water)

available as a medium in which relative movement of the nonaqueous constituents could occur. A study of curves for the relation between relative vapor pressure and moisture content of the fresh and stale samples should answer the question as to the possible role of water in the staling process.

In the present studies, relative vapor pressure–water content curves have been obtained on fresh and stale bread crumb prepared from the same loaf of bread and in such a manner as to minimize hysteresis effects other than those resulting from the staling process itself. Similar curves have been obtained on retrograded and nonretrograded samples of whole starch and upon the amylose fraction of starch. Certain conclusions regarding the possible roles played by water and by starch in the staling process are reached on the basis of the experimental findings.

Methods

Three methods for measuring the relative vapor pressure of the water in bread crumb and starch at various moisture contents were used in this investigation, namely, (a) the isotenoscope technique of Smith and Menzies (1910), (b) equilibration over sulfuric acid solutions of known composition, and (c) a modification of the Brunauer and Emmett (1934) method for measuring the adsorption of a gas on a solid.

The isotenoscope is simply a bulb, into which a sample of moist solid can be introduced, connected through a U-tube to a vacuum pump. An indicator oil is present in the bottom of the U-tube, and a mercury manometer is open to the line beyond the U-tube. In measuring the relative vapor pressure by this method, a sample of the dried, finely powdered solid is moistened with a small amount of water, placed in the isotenoscope bulb which is held in a thermostat at the desired temperature, and subjected to a strong vacuum which causes a part of the water to vaporize and sweep out all other gases from the bulb past the oil in the U-tube. The bulb and its contents are allowed to regain the temperature of the bath, and the vacuum in the line is kept at such a value as to prevent any gas from returning to the bulb. After a short time, during which equilibrium is attained between the adsorbed water in the sample and the water vapor in the bulb, the levels of the oil in the two legs of the U-tube are brought to equal heights and the pressure in the line, which equals the water vapor pressure in the bulb, is read on the manometer. The ratio of this pressure to that of pure water at the temperature of the experiment is the relative vapor pressure of the water in equilibrium with the moist solid. The vacuum is then released, the sample removed into a

weighing bottle which is quickly stoppered, and the wet weight of the sample determined. The sample is then dried for one hour at 130°C in a forced draft oven and the dry weight of the sample found. The difference between the wet and dry weight is a measure of the moisture content in equilibrium with the particular relative vapor pressure determined. The water content per gram of dry sample at that relative vapor pressure is thus a point on the relative vapor pressure–water content curve. The method allows for a quick determination of relative vapor pressures in the range between 0.4–0.8 but is not dependable below 0.4 because of the slowness of attainment of equilibrium, or above 0.8 because of difficulty in obtaining accurate enough pressure measurements in this region where the relation between water content and relative vapor pressure is changing rapidly. Each point to be determined on the curve requires the use of a different sample of solid, which conceivably might introduce some sampling errors.

The second method, that of equilibration of samples of the bread or starch over sulfuric acid solution of known relative vapor pressures, is one which has been used by numerous workers in investigations of this kind. It has the disadvantage that long times (as much as two weeks) are needed for equilibrium to be attained. During this time the temperature must be kept constant in all parts of the closed vessel used to contain the samples and the solution. In earlier studies (Geddes *et al.*, 1946) it was observed that, when the water content of bread crumb was above 20%, staling of the crumb could be detected. For this reason it is doubtful that equilibrium would be attained in this method short of the time required for fresh crumb to change at least partially to stale crumb for samples in which the equilibrium moisture content was greater than 15–20%, and that, therefore, this method could not be depended upon to detect difference between fresh and stale crumb in the relative vapor pressure range lying above this value of water content of the crumb. In the present studies this method was used to supplement the values obtained with the isotenoscope in the R.V.P. range below 0.4.

With a third method, employing a modification of the adsorption apparatus of Brunauer and Emmett, an attempt was made to obtain, on a single sample of the adsorbent (bread or starch), the entire R.V.P.-water content curve for relative vapor pressures varying from 0 to 0.9. Water vapor could be metered into a chamber containing the adsorbent and, after equilibrium had been attained, its pressure determined. Because of many difficulties, not all of which have as yet been overcome, about the only important observation obtained as a result of the use of this method is that, at very low moisture content of the adsorbent (R.V.P. values less than 0.3), very long times

(24 to 36 hours) were required for equilibrium to be attained, while above relative vapor pressures of 0.4, equilibrium was very closely approached in about one-half hour.

Materials

Preparation of Bread Samples. The first part of this study involved the preparation and preserving of bread crumb in relatively fresh and stale states. Farinograph and crumb swelling tests showed that the rate of staling of bread was negligible for bread crumb held below 20% moisture content. The observation of Katz (1928) that freshly baked bread does not stale when held above 60°–70°C was confirmed and this together with the above observation led to a simple procedure for preparing relatively fresh samples of bread crumb. The crumb of freshly baked bread, while still hot, was placed in a vacuum oven and dried overnight at 60°C. In the dry state it did not stale.

Relatively stale bread crumb could be prepared by allowing freshly baked bread to stale in containers at 4°C and then drying the crumb to a low moisture content at room temperature. Under these conditions no reversal of the staling took place.

Both the relatively fresh and stale samples could be stored in desiccators without apparent change for an indefinite period of time.

TABLE I

EFFECT OF DRYING BREAD ON THE DEGREE OF STALENESS

Sub-sample	Age after baking	Farinograph consistency[1]	Crumb swelling[2]	Crumb moisture
	Days	*B.U.*		%
1. Fresh crumb	0.04	585	4.43	44.3
2. Fresh crumb dried at 70°C	1	600	4.16	1.6
3. Fresh crumb dried at 70°C and stored in dry state	8	600	4.02	1.6
4. Stale crumb	6	—	2.88	35.8
5. Stale crumb after drying	6	—	2.91	6.1

[1] Fuller (1938) and Geddes *et al.* (1946).
[2] Katz (1912, 1934a), Cathcart and Luber (1939), and Geddes *et al.* (1946).

Table I gives some of the results of the work on the preparation of these samples and illustrates that the dried samples so prepared were indeed relatively fresh or stale, respectively, as indicated by crumb swelling and plasticity (Geddes *et al.*, 1946) measurements made subsequent to the drying procedures.

Preparation of Starch Samples. The following procedure was employed for the preparation of retrograded and nonretrograded starch, and later for the preparation of corresponding samples of amylose. A 3% starch suspension was gelatinized at 70°C on a water bath and

divided into two portions (one each for the preparation of the retrograded and nonretrograded starch). The portion used for the preparation of nonretrograded starch, while still above 65°C, was transferred immediately to a vacuum oven and dried for 5 hours at 70°C. This material dried to thin, brittle sheets, and it was necessary to grind it to a coarse powder in a Wiley mill before using it in adsorption tests. The nonretrograded starch dried in this way undergoes retrogradation to a much lower extent than when dried at lower temperatures after freezing (see below). As it contained less than 10% moisture, it could be stored in a desiccator for a considerable length of time without change (Katz, 1928). The portion used to prepare retrograded starch was allowed to cool to room temperature and was placed in an icebox at 4°C for 2 to 3 days. This treatment was usually followed by freezing the solution at −10°C. After thawing, the material was dried in air or in a partial vacuum at room temperature. The retrograded starch prepared in this manner contained less than 10% moisture and could be stored in a desiccator.

Preparation of Amylose Samples. Starch amyloses were prepared from both potato and wheat starches by the method of Schoch (1942) using n-butanol as the fractionating agent. As recommended by Schoch, the wheat starch was defatted prior to the amylose preparation by refluxing five times with 85% methanol (Schoch, 1942a). The potato starch did not require a similar treatment since its fatty acid content is already extremely low (Schoch, 1942a). The amylose-butanol complexes from the fractionations were stained with iodine and appeared as blue rosettes when viewed under the microscope. The amyloses from the fractionations were washed (with vigorous stirring) three times with methanol, once with ether, and then dried for 2 hours at 65°C in a vacuum oven. This postfractionation vacuum treatment was carried out in order to remove, as completely as possible, any readily volatile nonaqueous substances from the amylose, since these would interfere in the isotenoscope method. The retrograded and nonretrograded amyloses were then prepared in the manner already described for the whole starches.

Results and Discussions

Studies on Bread Crumb. In preliminary trials with the isotenoscope, equilibrium between the moisture adsorbed by bread crumb and and its vapor (within the range of R.V.P. values 0.4 to 0.8) was closely approximated within one-half hour. At R.V.P. values below 0.3, equilibrium was not attained even within a 3-hour period. To obtain the R.V.P. values below 0.3, equilibrium measurements were made with the bread crumb exposed over the appropriate sulfuric acid solution.

The first experiment consisted of an investigation of the water-binding capacity of fresh and stale bread crumb. The bread crumb used in this experiment was prepared from fresh white bread baked in the laboratory according to the standard procedure of the A.A.C.C. (*Cereal Laboratory Methods*, 4th ed., 1941). The baking formula contained: 100 g. flour, 5 g. sucrose, 1.25 g. salt, 0.125 g. calcium propionate (to inhibit mold growth), 3 g. yeast, and water as required. The relative vapor pressure–water content curves obtained on these samples are shown in Figure 1.

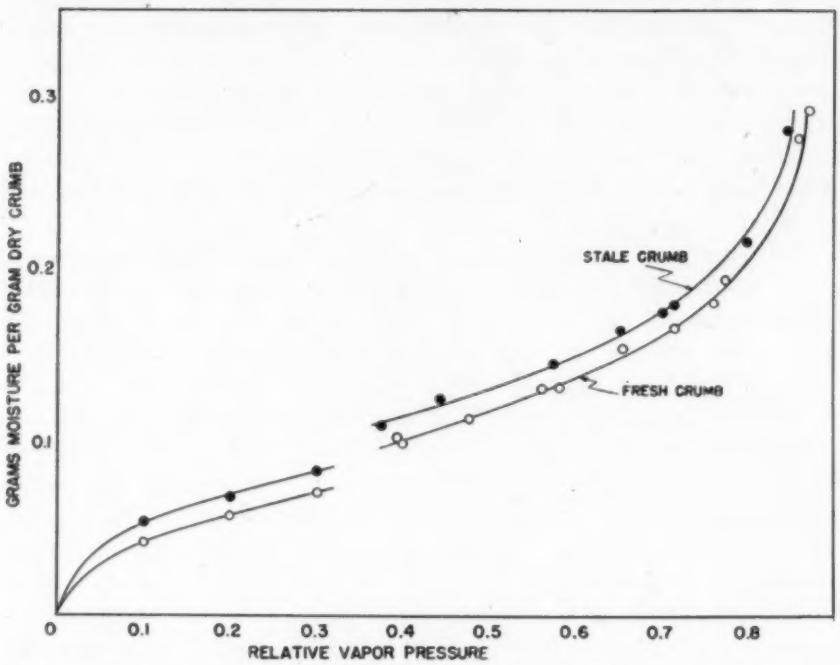

Fig. 1. Relative vapor pressure–moisture content curves for fresh and stale bread crumb. (Isotenoscope determinations conducted at 24.6°C. Equilibrium values over sulfuric acid solutions conducted at 25°C and determined after 22 days.)

A discontinuity is apparent in the curves obtained by the two methods. This is undoubtedly due to a failure to attain absolute equilibrium, between moisture deep inside the crumb particle and moisture at the surface, within the 30-minute interval employed in the isotenoscope method. The difference in the amount of moisture retained at a given relative vapor pressure by different materials (in this case the difference between moisture retained by stale versus fresh crumb) is, however, nearly the same when determined by either method. Since, in the present research, the difference in water binding is of importance, rather than the absolute amount of water bound, the isotenoscope

method is considered to yield results with sufficient accuracy for our purpose, even though these values may not be absolute values. The greater speed of the determination makes this method preferable in the present study, particularly in the higher relative vapor pressure range.

Two observations which are of importance to the problem under investigation are indicated in the curves of Figure 1. The first is to the effect that the differences in water binding by the fresh and stale crumbs are so small as to be insignificant, on the basis of the total water present in bread, as a possible cause of the structural changes which are observed to occur. It can be definitely concluded that these structural changes are not due to a significant decrease in the amount of water which is free to act as a medium of dispersion for the nonaqueous constituents of the bread. The increase in structure during the staling process must, therefore, be due to the formation of some type of cross linkage between the nonaqueous constituents of the bread.

The second point of importance which is indicated by these data is that, while the additional amount of water bound by the stale crumb is very small, it is, nevertheless, definite. It would be expected that, with the formation of cross linkages between the nonaqueous elements of the bread, there should occur a concomitant decrease in the surface area of these components remaining exposed to the water. This should normally result in a *decrease* in the amount of water bound per unit weight of dry bread substances. The fact that the observed change is an increase in water binding during staling suggests the possibility that the cross linkages formed actually occur *through* a water molecule as a part of the bridge or bond between the other components. Such a hypothesis would be strengthened if it could be shown that this observed increase in water binding is proportional to the extent of cross binding taking place in the bread, or bread component, during staling.

Questions which immediately present themselves with respect to this slight but definite increase in water binding resulting from staling of the crumb are: (a) May this increase actually be due to some other conditions of the experiment rather than to a true difference in water binding? (b) What substance of the bread is responsible for this change in water binding, if such it proves to be? (c) Can this slight increase in binding of water result from hysteresis effects other than those associated with the staling process?

This observed difference could conceivably be explained on the assumption that the stale bread crumb had associated with it some nonaqueous substances which were nonvolatile at ordinary temperatures but which might be lost at the temperature of the final drying

during the preparation of the "fresh" bread crumb. The stale crumb had been dried at room temperature only, while the fresh bread crumb had been dried in a vacuum oven at 60°C. Another possibility could be the presence of a greater amount of low molecular weight water-soluble constituents in the fresh crumb than in the stale crumb. Evidence was obtained which showed that neither of these possible factors was operating, and thus the conclusion that stale bread crumb binds a slightly greater amount of moisture than fresh crumb could be drawn with considerable assurance. The methods of obtaining this evidence follow.

To investigate the first of the above possibilities, the stale crumb was treated in a manner which would remove any such nonaqueous volatile substances (if any were present) without causing changes in the degree of staleness of the crumb. This was accomplished by heating a sub-sample of the original lot of stale crumb at 70°C for 5 hours in a forced draft oven. The risk of refreshening the original stale crumb during this process was negligible due to the fact that its moisture content was considerably less than 10% (Katz, 1928, 1934; Katz and Weidinger, 1934; and Geddes *et al.*, 1946). R.V.P.-water content data were obtained for the stale crumb prepared in this manner and, when plotted, all of the points were found to lie on the R.V.P.-water content curve for the original stale sample. This result demonstrated that there were no nonaqueous volatile substances (at least, none that were not also associated with the fresh crumb) which could cause the stale crumb to appear to contain more moisture at any given relative vapor pressure than the fresh crumb.

The second aforementioned condition either did not exist, or did so to such a small extent that it could not be detected, as was indicated by the fact that aqueous extracts of fresh and stale crumb (1 g. of crumb was extracted with 40 ml. of water at 24.65°C for 45 minutes with shaking) gave the same mean freezing point of −0.048°C. This means that the activity of the water present in the fresh and stale crumb was equally depressed by any nonaqueous low molecular weight water-soluble substances which were present.

Studies on Starch. It was important to determine if the small observed differences in water-binding capacity of fresh and stale bread crumb were due to some change which the starch, as one important component of bread, had undergone during the staling process. Katz (1912, 1914, 1915, 1928) proposed that the development of crumbliness, increase in crumb rigidity, and decrease in swelling power of the crumb were due to the retrogradation of starch. This hypothesis could be tested by comparing the relative water-binding capacities of retrograded and nonretrograded starch with the corresponding values for

stale and fresh bread crumb. This comparison was the first objective of the work on starch.

Water content-R.V.P. curves (Figure 2) were determined by the isotenoscope method for a sample of ungelatinized commercial wheat starch and for retrograded and nonretrograded samples derived from it. The moisture-binding capacity was the greatest for the retro-

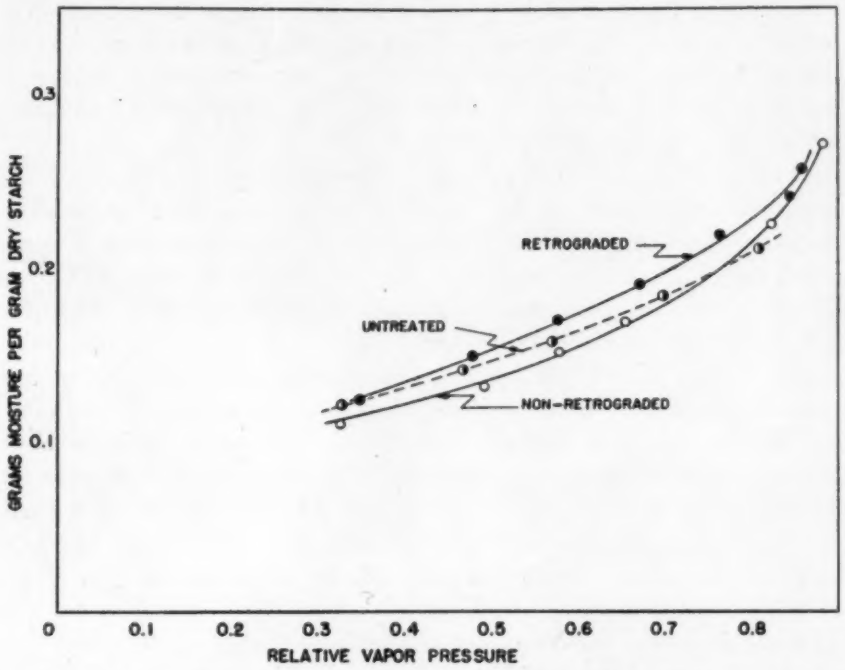

Fig. 2. Relative vapor pressure–moisture content curves for undefatted wheat starch. (Isotenoscope determinations conducted at 24.6°C. Curve 1—retrograded starch; curve 2—untreated starch; and curve 3—nonretrograded but gelatinized starch.)

graded sample and least for the nonretrograded starch, the difference amounting to approximately 0.015 g. of water per gram of dry starch over the range of relative vapor pressures studied. The moisture-binding capacity of the untreated starch was intermediate between that of the derived retrograded and nonretrograded samples. On the assumption that starch in stale bread is retrograded, and that starch of fresh bread is in the nonretrograded condition, the results indicate that the difference in the moisture-binding capacity of stale and fresh bread may be due at least in part to the retrogradation of the starch in the bread.

Investigations of Possible Hysteresis Effects During Sample Preparations. The preparations of stale crumb and retrograded starch,

up to this time, had not usually been subjected to a final oven drying previous to the determination of the R.V.P.-water content curves as had been the case with fresh crumb and nonretrograded starch. That the difference observed in water-binding capacities between stale and fresh bread (or between retrograded and nonretrograded starch) was due to the staling (or retrogradation) process alone was indicated by the one experiment on postheated stale crumb already described wherein the postheated and unheated stale crumb gave coincident R.V.P.-water content curves. An investigation of this kind was repeated, however, making certain that hysteresis effects other than those due to staling and retrogradation were held at a minimum. Defatted wheat starch was used in this study.

For this experiment it was necessary to prepare retrograded and nonretrograded starch samples which would have, as nearly as possible, the same previous treatment. The procedure employed was as follows: a 3% starch suspension was gelatinized at 90°C, cooled to 60°C, and divided into two portions. The nonretrograded fraction was prepared from one of the portions (while still at 60°C) by adding a large excess of methanol. The resulting precipitate was collected by vacuum filtration and dried at 55°C at reduced pressure. A saturated solution of sodium bromide in the drying chamber maintained a relative vapor pressure of approximately 0.5 at the drying temperature. The humid atmosphere prevented the starch from drying to a hard, brittle film but allowed it to reach a water content low enough to retard subsequent staling. Retrogradation of the remaining portion of the gelatinized starch was completed after two days' exposure at 4°C. This solution was frozen at −10°C, thawed, and was then added to three volumes of methanol. The purpose of the methanol was to approximate the conditions employed in the preparation of the nonretrograded starch. The precipitated starch was collected on a filter and dried in the manner described above. Treatment with the methanol had reduced its water content to a low enough value so that the subsequent heat vacuum treatment should not cause it to refreshen. Both the retrograded and nonretrograded starches were ground in a Wiley mill (No. 60 screen) and then conditioned to a relative vapor pressure of 0.3 at 25°C.

The relative vapor pressures of the retrograded and nonretrograded defatted whole wheat starches at various moisture contents were determined by the sulfuric acid method at 25°C. The samples were weighed daily after the fourth day. The change in moisture content of the samples was negligible between the seventh and eighth days, and the moisture content became constant by the end of the ninth day.

TABLE II

MOISTURE CONTENT-R.V.P. RELATIONSHIPS IN DEFATTED WHEAT STARCH
(Tests conducted over sulfuric acid solutions at 25°C)

	Moisture per gram of dry starch at the given relative vapor pressure		
R.V.P.	0.1	0.2	0.3
	g.	g.	g.
Retrograded defatted wheat starch	0.0645	0.0888	0.1050
Nonretrograded defatted wheat starch	0.0595	0.0835	0.1005

These results, given in Table II, show that similar relative relationship exists between the water-binding capacity of the retrograded and nonretrograded starches prepared in the above manner as was observed with the starch and bread crumb samples used in the previous experiments. The observed difference between the water-binding capacity of retrograded and nonretrograded starch is 0.005 g. of water per gram of dry starch. The difference is not as large as would be expected on the basis of the previous data. The discrepancy is undoubtedly due to the differences in the methods of preparing the samples. No independent method was applied to compare the differences in relative degrees of retrogradation between the starch samples prepared by the different methods. This experiment does show, however, that if retrograded and nonretrograded starches have had, as nearly as possible, the same previous history, a slightly greater amount of moisture will still be bound by the retrograded material. This difference thus must be considered as significant.

Studies on Amylose. There remained the desirability of obtaining some information concerning the question as to whether or not the observed small increase in water binding which occurs during the staling process in bread and the retrogradation process in starch parallels in amount the degree to which cross linking takes place during the process.

It was reasoned that the degree to which cross linkage could take place, per unit of dry bread or starch substance, before the components were immobilized to such an extent that further formation of such links would be prevented, would be a function of the intimacy with which the components so involved might be able to approach each other. The branched nature of the amylopectin component of starch would tend to prevent it from forming as great a number of cross linkages per unit weight as would the straight-chained amylose component, particularly when the latter was not impeded in the process by the pres-

ence of amylopectin or other components.[4] It was reasoned, therefore, that, per unit mass of solid component, amylose should form a greater number of cross linkages during retrogradation than would whole starch, and whole starch should exceed bread crumb in this regard. If the increase in the binding of water should be proportional to the number of cross linkages formed, then we should find that the difference in water-binding capacity of retrograded and nonretrograded amylose ought to be greater than the corresponding value for retrograded and nonretrograded whole starch. This relationship was therefore investigated.

Measurements made on retrograded and nonretrograded wheat and potato amyloses in the isotenoscope demonstrated that retrograded potato amylose bound about 0.035 g. more water per gram of dry amylose than the corresponding nonretrograded amylose. Retrograded wheat amylose bound about 0.025 g. more moisture per gram than the corresponding nonretrograded wheat amylose. As already mentioned retrograded whole wheat starch bound only 0.015 g. more water per gram than nonretrograded wheat starch, and the corresponding figure for stale versus fresh bread crumb was 0.010 g. per gram. It is clearly indicated that the difference in water-binding capacity of stale versus fresh crumb and of retrograded starch versus nonretrograded starch is a function proportional to the degree of retrogradation, i.e., cross linking, which the starch, whether alone or present as a constituent of bread crumb, is able to undergo under the circumstances of the experiment. This evidence, while inconclusive, supports the hypothesis that cross linkages formed during staling of bread and retrogradation of starch are accompanied by a strong fixation of some water molecules in the region of the bond. This could mean that the bond actually occurs through a water molecule or it could mean that where the bond is formed a new region is inaugurated upon which water is strongly adsorbed. That the strength of binding of this water is high, but weaker than a true chemical bond, is indicated by the fact that it will only be removed from the starch at a relative vapor pressure somewhere below 0.05 but above zero R.V.P.

Conclusions

The role of water in the bread-staling process, insofar as the development of a rigid structure in the bread is concerned, is primarily that of a medium in which rearrangement of starch molecules can occur to positions with respect to one another such that cross linkages

[4] It may well be pointed out, however, that only a few crosslinks between the amylopectin components could result in a greater increase in structure than would many crosslinks involving only the amylose component. The question of importance in this experiment is not that involving the amount of structure but the relative number of cross linkages.

(through hydrogen bonds, presumably) can form between them. The structure generated during staling is due to these cross linkages between nonaqueous elements of the bread and not due to any appreciable increase in the binding of water by these nonaqueous elements.

It appears from these experiments, however, that a very small amount of water is directly involved in the crumb-staling process. The increase in the water bound when bread crumb stales (or when starch retrogrades) is too small (approximately 1–3 molecules for 10 glucose units) to account for the increase in crumb rigidity on the assumption that water becomes no longer free to act as a plasticizer. Rather, the evidence favors the hypothesis that the increase in crumb rigidity is due to the formation of cross linkages between starch molecules by hydrogen bonds which in some way involve a small number of water molecules. The fact that straight-chained amylose acquires during retrogradation a somewhat higher percentage increase in moisture-binding capacity than whole starch substantiates this point of view.

Summary

Using an isotenoscope method and a method involving attainment of equilibrium over sulfuric acid solutions of known relative vapor pressures, the relative vapor pressures of water present at various moisture levels in stale and fresh bread crumb, in retrograded and nonretrograded starches, and in retrograded and nonretrograded amyloses were determined.

The relative vapor pressure–moisture content curves, so obtained, indicated that a small but significant increase occurs in the water-binding capacity of stale crumb over that of fresh crumb, and in retrograded over that of nonretrograded starches and amyloses. This increase in water-binding capacity for bread crumb amounts to about 0.01 g. of water per gram of dry crumb. The corresponding figure for whole wheat starch is of the order of 0.015 g., for wheat starch amylose 0.025 g., and for potato starch amylose 0.035 g.

The small increase in water-binding capacity of bread crumb during staling is of no importance as a means of increasing the structural properties of bread through the mechanism of removal of water in its role of a plasticizing medium. The structure developed in staling is due to the formation of cross linkages between the nonaqueous elements of the bread substance. The small amount of water additionally bound during the process indicates, however, that the cross linkages formed may involve some water molecules. The observation that a similar process occurs in the retrogradation of starches and amylose is considered to indicate that these elements are the ones responsible

for the structural evidences of staling in bread crumb. These effects are considered to be in excess to the usual hysteresis effects observable with starch, etc., resulting from simple drying or rehydration processes.

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GRAIN STORAGE STUDIES. VII. INFLUENCE OF CERTAIN MOLD INHIBITORS ON RESPIRATION OF MOIST WHEAT[1,2]

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(Received for publication July 15, 1947)

The rapid increase in the respiration of seeds at moisture contents in equilibrium with atmospheric relative humidities in excess of about 75%, described by Bailey and Gurjar (1918) and Bailey (1940) as an inherent activity of the seeds themselves, is now known to be due largely to the growth of molds on and in the seed. That molds are the primary cause of heating and deterioration of various kinds of stored seeds at moisture contents where molds can grow has been shown by a number of workers, including Gilman and Barron (1930), Milner and Geddes (1946, 1946a), Milner, Christensen, and Geddes (1947), Nagel and Semeniuk (1947), and Semeniuk, Nagel, and Gilman (1947). In a comprehensive review of the literature on the deterioration of corn in storage, Semeniuk and Gilman (1944) state that "the conditions under which deterioration occurs and the changes which follow its initiation indicate that it is primarily a biological decomposition."

In a preliminary attempt to estimate the inherent respiration of moist wheat independent of that due to molds, Milner (1946) tested 11 sulfonamide compounds as mold inhibitors, and, of these, sulfanilamide proved to be the most effective. In the present studies, sulfanilamide and seven other compounds, to be enumerated later, were tested, the primary aim being to inhibit the growth of molds so that the respiration of the wheat itself could be measured at different moisture contents.

Materials and Methods

More than 100 compounds[6] were given various preliminary screening tests as fungistatic agents on moist wheat. The wheat was con-

[1] Cooperative investigation of the Divisions of Agricultural Biochemistry and of Plant Pathology and Botany. Paper No. 2350, Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] This investigation was aided by a grant from Wallace and Tiernan Company, Inc., Newark, New Jersey.

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[6] The following compounds were tested:

acetyl thiourea
alkyl-dimethyl-benzyl ammonium chlorides (Zephiran, or Zephiran chloride)
alkyl-aryl-sulfonate (Nacconol FSNO)
alkyl thiourea
ammonium sulfamate
p. aminoacetanilide
4-aminoazobenzene 4-sulfonic acid
β-aminobenzothiazole
p. aminobenzoic acid
α-amino butyric acid
amino guanidine sulfate
1-amino 2-naphthol 4-sulfonic acid
1-amino 8-naphthol 4,6-disulfonic acid
p. aminophenol
o. aminophenol p. sulfonic acid
2-aminothiazole
benzene sulfonamide
benzene sulfon-N-dichloramide (Dichloramine B
benzoic acid
borax
boric acid
butane monosulfonamide

ditioned to moisture contents between 16% and 25%, then the compounds were, in most cases, applied as dusts, in concentrations of one part by weight of dust to from 100 to 1000 parts by weight of wheat seed. The treated wheat was stored at room temperature in stoppered 250 ml. Erlenmeyer flasks, or in 4 oz. screw-capped medicine bottles, and examined at various intervals up to 30 days. If molds were visible on the treated seed, no further tests were made, but if the grain appeared to be relatively free of molds, 50 to 100 seeds were surface disinfected and placed on nutrient agar to determine the amount of internal mold infection. Similar numbers were placed on agar, moist filter paper, or sterile sand, to determine viability, and a sample was analyzed for fat acidity, which Milner, Christensen, and Geddes (1947) have shown to be a good indicator of mold development.

Of the compounds tested in this way, relatively few prevented visible development of molds on the moist seed. Even some compounds known to be toxic to certain fungi under certain conditions, such as calcium propionate, "chloramine B," "Dithane," the two

calcium propionate
calcium undecylenate
carbon tetrachloride
chlorazene (Chloramine T)
chloroform
copper 8-hydroxyquinoline

dichloro-diphenyl-trichloroethane (DDT)
dichloroethylene
2,3-dichloronaphthoquinone-1,4 (Naugatuck 604)
2,4-dichlorophenoxyacetamide
2,4-dichlorophenoxyacetic acid
dioctyl ester of sodium succinic acid (Aerosol OT)
diphenyl
diphenyl thiosemicarbazide
diphenyl thiourea
diphenyl urea
di-tertiary butyl diperphthalate
"Dithane A-10" (an experimental product consisting of 85% disodium ethylene, bisdithiocarbamate hexahydrate and 15% of inert ingredients)

epichlorohydrin

hexachlorobenzene
hexachloroethane
Hyamine
hydroquinone
8-hydroxyquinoline
8-hydroxyquinoline sulfate
8-hydroxyquinoline 5-sulfonic acid

isobutylene oxide
isopropyl glycidyl ether

mercaptobenzothiazole
methyl guanidine sulfate
methyl hydrazine sulfate
2-methyl, 1,4-naphthoquinone

1,4-naphthoquinone

perchloroethylene
α-phenoxy propionic acid
4-phenyl thiosemicarbazide
phenyl thiourea
phthalyl sulfathiazole
propane 1,3-disulfonamide
propylene glycol
propylene oxide

quinone

sodium alkyl aryl sulfonate (Nacconol NR)
sodium anthroquinone β-sulfonate
sodium benzoate
sodium N-chlorobenzenesulfonamide (Chloramine B)
sodium propionate
sucrose
sulfamic acid
sulfanilic acid
sulfadiazine
sulfaguanidine
sulfamerazine
sulfamethazine
sulfanilamide
sulfapyrazine
sulfapyridine
sulfaquinoxiline
sulfasuxidine
sulfathiazole
sulfur

tert. butyl hydroperoxide
tert. butyl perbenzoate
tetrachloroethylene
tetramethylthiuramdisulfide
tetrachloro-para-benzoquinone (Spergon)
thioacetamide
thioacetanilide
thiodiphenylamine
thiobenzanilide
thiomalic acid
thiosemicarbazide
thiourea
toluene
o. toluene sulfonylamide
p. toluene sulfonylamide
p. toluene sulfonylmethylamide
p. toluene sulfonyldimethylamide
p. toluene sulfonyl n-butylamide
trichloroethylene
2,4,5-trichlorophenoxyacetic acid

undecylenic acid
urea

zinc stearate
zinc undecylenate

"Nacconols," and "Spergon," did not prevent the visible development of molds on the wheat seed treated with them. In some cases a given fungicide inhibited certain molds, but not others, or inhibited exterior development and spore production of one or more molds, while not inhibiting the growth of molds within the seed. Some of the compounds that inhibited molds also killed the seed.

On the bases of low toxicity to the seed and fair to high toxicity to molds, eight compounds seemed worthy of further testing, namely: 2-aminothiazole, benzene sulfonamide, calcium propionate, chloramine B, 8-hydroxyquinoline sulfate, p-aminobenzoic acid, sulfanilamide, and thiourea. These compounds, as fine powders, were thoroughly mixed into a sound sample of Regent wheat (a hard red spring variety) testing 94% germination. The seed was conditioned to 20% moisture, and the compounds were added at the rate of 1 part per 1000 parts by weight of the damp grain.

In a separate series of experiments thiourea at a concentration of 1.0% of the damp seed weight was dusted on Regent wheat conditioned to various moisture contents from 14.2% to 35.5%. Untreated samples at each moisture were included.

The respiration was measured over a 10-day period with the apparatus and technic described by Milner and Geddes (1945) in which continuously aerated samples of the wheat were held at 30°C and the appropriate relative humidities to maintain the desired moisture contents. The daily accumulations of effluent air were analyzed with a Haldane-Henderson gas analyzer. Care was taken to avoid concentrations of carbon dioxide in the respirometers which might inhibit respiration. Moisture contents using the two-stage, air-oven method, and fat acidity were determined according to the technics described in *Cereal Laboratory Methods* (4th ed., 1941). To determine mold populations at the end of the respiration trials, small lots of seed were ground in an intermediate Wiley mill equipped with a 40-mesh sieve. The mill was first flushed with alcohol to disinfect it and the air-dried samples were ground in succession, beginning with the one of lowest respiratory activity and progressing to the highest. The meals so obtained were cultured at various dilutions in malt-salt agar according to the technic described by Christensen (1946). Germination tests of the seed were made by the Minnesota State Seed Testing Laboratory.

Results and Discussion

Evaluation of the Various Fungistatic Agents by Measuring the Respiration of Treated Wheat. The respiratory rates of the control and of the samples treated with the eight fungistatic agents at a concentration of 0.1% are presented in Figure 1.

The respiratory rates of all the samples, both treated and untreated, were virtually identical during the first two days of the trial, the approximate time required for the molds to begin vigorous growth on wheat at 20% moisture. The shape of the respiration curve for the control sample was essentially similar to a curve of microbiological growth. The effectiveness of the compounds in inhibiting mold growth was roughly inversely proportional to the carbon dioxide production of the treated wheat samples after 7 to 10 days. With thiourea

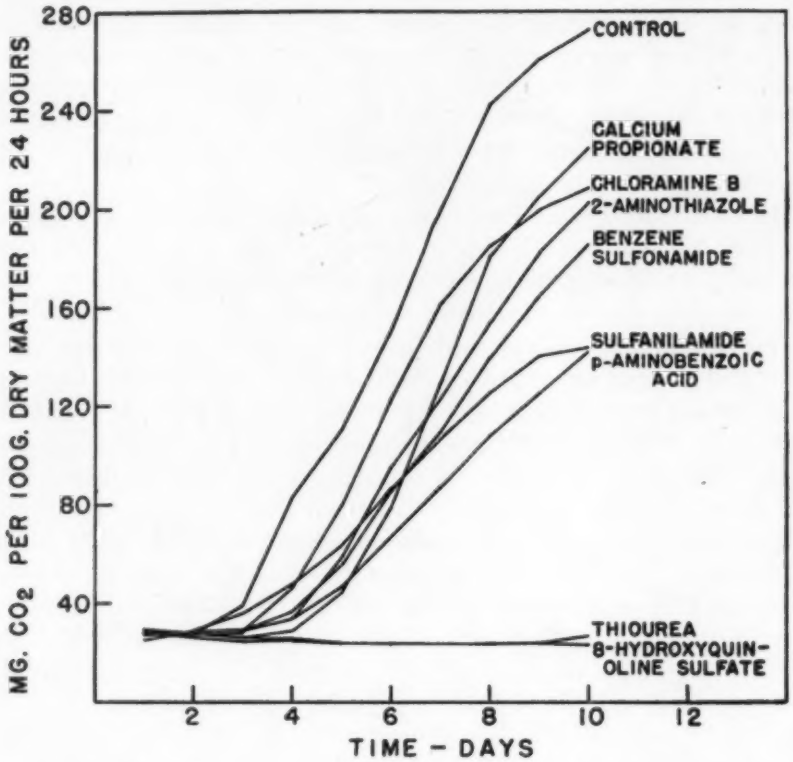

Fig. 1. Influence of time and various mold inhibitors on the respiratory rate of Regent wheat at 30°C containing 20% moisture. The inhibitors were used at a concentration of 1 : 1000.

and 8-hydroxyquinoline sulfate, the respiration remained practically constant (24 to 30 mg. carbon dioxide for 24 hours) for 10 days. This, apparently, approximately represents the natural respiration of this sample of wheat under the conditions of the test.

At the end of the trials, the samples were tested for fat acidity and germination, and the mold population of several samples was determined. These data are presented in Table I, the samples being listed in decreasing order of respiratory rate on the final day of the trial.

TABLE I

INFLUENCE OF VARIOUS FUNGISTATIC AGENTS ON RESPIRATORY RATE, FAT ACIDITY, GERMINATION, AND MOLD GROWTH IN WHEAT CONTAINING 20.0% MOISTURE

(Samples analyzed after completion of 10-day respiration trials at 30°C)

Treatment	Respiratory rate[1]	Fat acidity[2]	Germination[3]	Mold colonies per gram
			%	
Control	273.0	77.0	18	6,950,000
Calcium propionate	224.8	91.7	15	4,320,000
Chloramine B	208.6	95.8	21	3,170,000
2-Aminothiazole	202.8	75.3	17	—
Benzene sulfonamide	185.5	52.0	27	—
Sulfanilamide	144.0	65.1	26	2,420,000
p-Aminobenzoic acid	143.7	60.1	49	—
Thiourea	26.3	15.2	92	22,000
8-Hydroxyquinoline sulfate	23.9	16.6	64	—

[1] Mg. CO_2 per 100 g. dry matter per 24 hours on 10th day of trial.
[2] Mg. KOH per 100 g. wheat, dry basis.
[3] Initial germination was 94%.

In general, final fat acidity and mold population decreased with decreased respiration, while the percentage of seed germination increased. The viability of the wheat treated with thiourea was not reduced. Some of the respiration of the sample treated with thiourea was apparently due to molds, since the molds did increase slightly. Although 8-hydroxyquinoline sulfate is an effective mold inhibitor, it was slightly toxic to the seeds.

The data for the daily respiratory quotients of each sample are given in Table II. The course of the respiratory quotient for the control sample was the same as that noted by Milner, Christensen, and

TABLE II

INFLUENCE OF VARIOUS FUNGISTATIC AGENTS ON THE RESPIRATORY QUOTIENT OF WHEAT CONTAINING 20.0% MOISTURE

	Respiratory quotient								
Day	Control	Calcium propionate	Chloramine B	2-Aminothiozole	Benzene sulfonamide	Sulfanilamide	p-Aminobenzoic acid	Thiourea	8-hydroxyquinoline sulfate
1	—	0.98	1.14	1.13	—	1.04	—	—	—
2	1.16	1.04	1.05	0.98	0.98	1.03	1.02	1.04	1.08
3	1.03	1.05	1.04	1.02	1.00	1.01	1.06	0.98	1.03
4	1.06	0.99	1.04	1.05	1.09	1.03	1.02	1.01	1.04
5	0.96	0.93	0.99	1.02	1.00	1.03	1.00	1.03	1.03
6	0.88	0.89	0.92	0.95	0.99	0.99	0.98	1.04	1.01
7	0.86	0.91	0.89	0.90	0.94	0.96	0.94	1.05	1.00
8	0.80	0.90	0.88	0.85	0.90	0.93	0.90	1.00	—
9	0.83	0.85	0.86	0.84	0.87	0.88	0.87	1.01	1.04
10	0.81	0.83	0.85	0.82	0.86	0.78	0.86	1.02	1.04

Geddes (1947) for wheat in this moisture range, in that a quotient of about unity in the first few days of the trial was followed by a progressive decrease with increasing mold growth and respiration to a value of about 0.8. The fungistatic efficiency of each of the compounds used was approximately proportional to the extension of the period during which respiratory quotients near unity were maintained. The daily respiratory quotients of the grain treated with thiourea and 8-hydroxyquinoline sulfate remained close to unity throughout the 10-day trial.

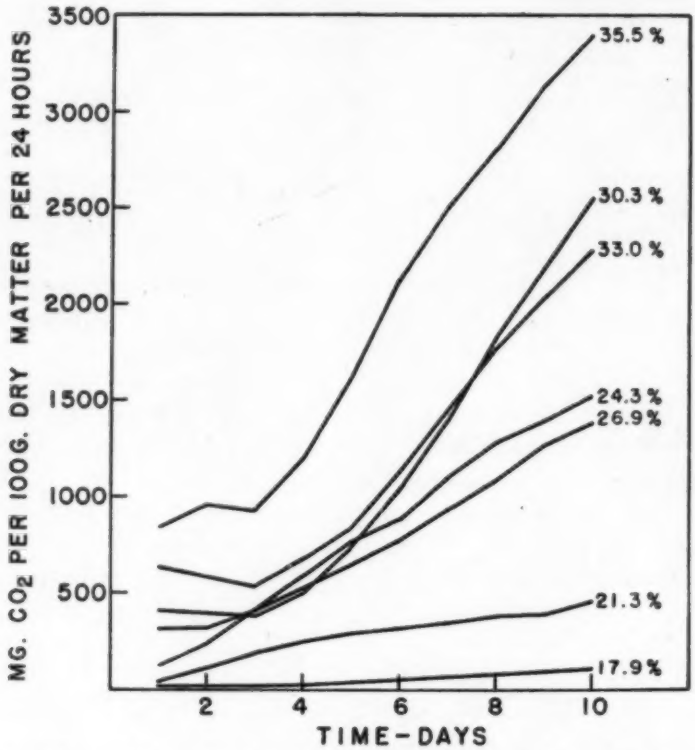

Fig. 2. Influence of time and moisture content on the respiratory rate of Regent wheat at 30°C. Respiratory rates of samples at moisture contents below 17.9% were too low to be indicated on this scale.

Influence of Moisture Content and Thiourea on Wheat and Mold Respiration. The fungistatic effectiveness of thiourea as well as its low toxicity to wheat suggested its use for estimating the inherent respiration of wheat at moisture contents up to that required for germination.

Because of the extremely large differences in respiratory rate of the treated and untreated samples, the data of each group are plotted separately. The respiratory behavior of the untreated samples is

shown in Figure 2 and is similar to that described by Milner, Christensen, and Geddes (1947) who showed that increases in respiration due to mold growth occurred in wheat at moisture contents in excess of 14.5%—the equilibrium moisture at 75% relative humidity. The optimum moisture for the growth of different molds varies, and a direct relationship between moisture content and respiratory rate would not be expected, especially at the higher levels where many

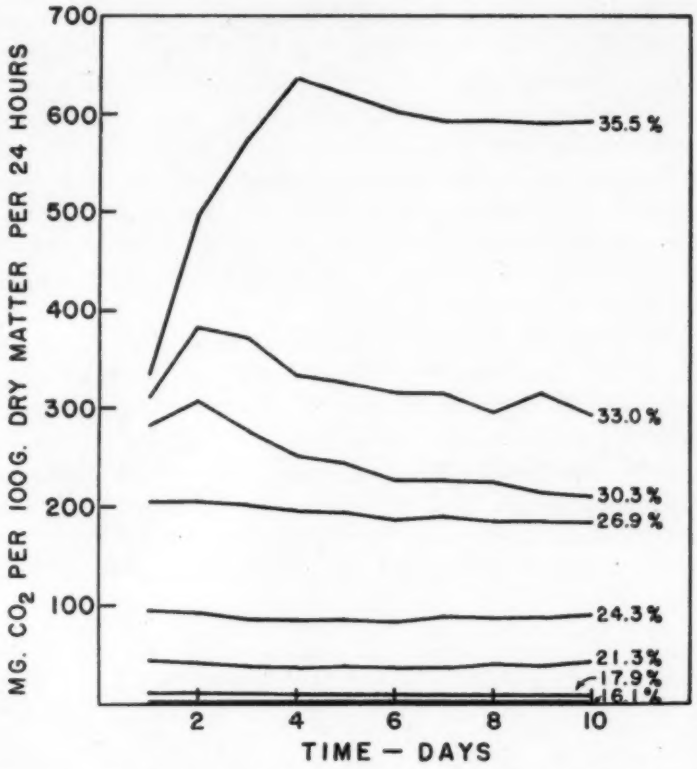

Fig. 3. Influence of time and moisture content on the respiratory rate of Regent wheat at 30°C, treated with powdered thiourea at a concentration of 1%.

species can grow. This may account, at least in part, for the irregularities shown in Figure 2. Thus, after the third day, the wheat at 24.3% moisture had a higher respiratory rate than that at 26.9%; from the fifth to the seventh days the rate for the wheat at 33.0% only slightly exceeded that for the sample at 30.3% moisture after which the rate for the lower-moisture sample became the greater.

The respiratory behavior of the thiourea-treated grain is shown in Figure 3. Except for its property of markedly inhibiting mold growth, and very slightly reducing seed respiration in the first few days, thi-

ourea had no significant influence on the respiratory characteristics of wheat at moisture contents as high as 24.3% and possibly as high as 26.9%, for the seeds respired at very nearly a constant rate throughout the trial. At still higher moisture values (30.3% and 33.0%), however, a brief initial increase in respiration was followed by a steady decrease. The sample containing 35.5% moisture showed a marked initial increase in respiratory activity which lasted for four days, but this trend was then reversed and the respiration declined as the trial progressed. At the end of the experiment many seeds in the sample containing 35.5% moisture had sprouts up to 2.5 cm. in length. Some seeds in the samples with 30.5% and 33.0% moisture also had germinated, a few seeds having sprouts from 0.5 to 1.0 mm. in length. Except for occasional slight swelling of the seed embryos in the grain at 26.9% moisture, no other samples showed evidence of germination.

These observations suggest that the initial increase in respiration shown by samples containing 30.3% and higher moisture, which were treated with thiourea, was due to the germination of the seeds. The subsequent decreases in respiration were apparently due to the death of some of the seeds. The almost linear respiratory values for the samples at moisture values below 26.9% suggest that thiourea is practically nontoxic to dormant seeds in the lower moisture range.

Additional evidence on the influence of thiourea in controlling mold growth and thereby reducing respiration and chemical and germinative deterioration appears in the data of Table III. That thiourea is fungistatic rather than fungicidal to the fungi here encountered is apparent since it caused no decrease in the original mold contamina-

TABLE III

INFLUENCE OF MOISTURE CONTENT AND THIOUREA TREATMENT ON RESPIRATORY RATE, MOLD GROWTH, FAT ACIDITY, AND GERMINATION OF WHEAT
(Samples analyzed after completion of 10-day respiration trials at 30°C)

Moisture	Respiratory rate[1]		Mold colonies per g.		Fat acidity[2]		Germination[3]	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
%							%	%
14.2	0.16	0.17	5,500	5,766	14.0	18.7	96	97
16.1	2.23	0.94	5,500	4,933	15.9	20.2	94	95
17.9	100.5	6.9	10,166	5,000	50.1	14.1	26	94
21.3	461.2	42.8	5,310,000	23,700	141.2	20.4	11	93
24.3	1512.8	90.6	6,710,000	34,800	92.4	38.5	5	81
26.9	1375.4	184.4	2,580,000	10,660	87.0	21.3	8	19
30.3	2539.4	209.6	65,000,000	77,500	231.4	55.9	—	15
33.0	2267.4	291.8	88,000,000	5,575	222.3	19.2	—	10
35.5	3394.7	592.6	95,000,000	50,000	265.2	58.0	—	2

[1] Mg. CO_2 per 100 g. dry matter per 24 hours on 10th day of trial.
[2] Mg. KOH per 100 g. wheat, dry basis.
[3] Initial germination was 94%.

tion of the seeds but was effective in preventing marked increases. The data do indicate, however, that molds increased slowly in the samples treated with thiourea, at moisture contents of 21.3% and above. The data for fat acidity show that thiourea, through its fungistatic action, inhibited chemical deterioration of the seeds. Seed viability was similarly protected from the toxic effects of mold growth. Viability of the seeds was maintained by thiourea at moisture values

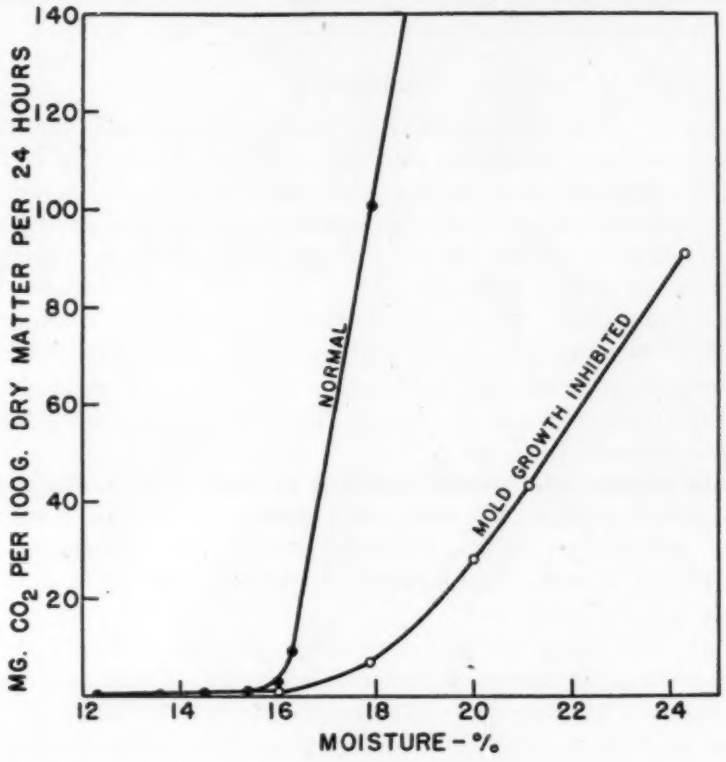

Fig. 4. Influence of moisture content on the respiration of normal wheat subject to mold growth and of the same wheat treated with thiourea at a concentration of 1% to inhibit mold growth, at 30°C. The respiratory trials were conducted for 10 days and the rates plotted for those for the 10th day.

up to and including 21.3%, whereas drastic decreases in germination occurred at moisture contents of 26.9% and above. The minimal moisture at which respiratory anomalies attributable to germination appear (between 24.3% and 26.9%) corresponds to the range of hygroscopic moisture in wheat in equilibrium with 100% relative humidity in the atmosphere at normal temperatures, which has been reported in the literature.

The relation between moisture content up to 24.3% and respiratory rate on the tenth day of the trial for both the normal and thiourea-

treated wheat samples is shown in Figure 4. Additional points on the curve for untreated wheat at moisture contents between 11% and 15% were taken from previous data of Milner, Christensen, and Geddes (1947). The inflection in the moisture-respiration curve for the thiourea-treated wheat occurs at a higher moisture value and is much less abrupt than that for the untreated wheat. The curve for the treated wheat apparently represents principally the influence of moisture content on the inherent respiration of the wheat at moistures up to those at which germinative processes are initiated.

Summary

More than 100 compounds were tested for fungistatic ability on wheat stored with a moisture content of 16% to 25%. Few of these effectively inhibited the growth of molds on or in the seed. Some compounds inhibited certain molds but not others, or inhibited the surface growth and spore production of certain molds without preventing the growth of the molds in the interior of the seed. This suggests that the effectiveness of a given compound in inhibiting the development of molds on or in moist stored seed of any kind can be ascertained only by determining the number and kinds of molds originally present, and their subsequent increase or decrease after the seed has been treated with the supposed fungicide.

Eight compounds extensively tested as moldicides on wheat with a moisture content of 20% were rated in order of decreasing value as follows: 8-hydroxyquinoline sulfate, thiourea, p-aminobenzoic acid, sulfanilamide, benzene sulfonamide, 2-aminothiazole, chloramine B, and calcium propionate.

Of the two most effective moldicides, thiourea was only slightly toxic to wheat at moisture contents below 24%, while 8-hydroxyquinoline sulfate reduced seed germination more than 30%.

Sound wheat stored at 30°C and at moisture contents above 16.1% was rapidly overgrown by molds. The increase in respiration and decrease in viability of the seed with increasing moisture content was proportional to the increase in molds.

Wheat treated with 1 part of thiourea to 100 parts by weight of moist seed respired at a nearly constant rate over a 10-day period, the viability decreased only slightly, and the molds increased only slightly up to a moisture content of 24.3%, although both molds and respiration had begun to increase at a moisture content of 21.3%. At moisture contents of 26.9% to 35.5% the seed viability was reduced.

The fat acidities of wheat treated with thiourea were markedly lower than those of the untreated grain after storage for 10 days at 30°C, especially at moisture contents above 17.9%.

The respiration of dormant wheat seed on which molds were inhibited (but not eliminated) increased gradually with increasing moisture content until the processes involved in germination became active.

The sharp increase in respiration of wheat at the so-called "critical" moisture content is caused by the respiration of molds on and in the seed.

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NOTE ON A RAPID METHOD FOR ESTIMATION OF MIXOGRAM AREA [1]

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(Received for publication August 25, 1947)

Mixogram areas have been used by Morris, Bode, and Heizer (1944) and Lamb (1944) as an index of quality of soft wheat varieties and by Johnson, Shellenberger, and Swanson (1946) to aid in the determination of uses for hard wheat flours. These workers obtained the area by tracing the mixogram with a planimeter which is expensive and rather tedious to use. It seemed likely that mixogram area might be more generally used if a simpler and more rapid method of measuring could be found.

Study of a large number of mixograms, made according to the procedure of Morris *et al.* (1944), revealed that there was a close correlation between area and the sum of two linear measurements; one being the height from the baseline to the center of the band at the point of minimum mobility and the other the height from the baseline to the center of the band at the 7-minute point. Plotting area against this sum showed that the relationship was linear and that most of the points fell close to a regression line.

The method was further tested on 344 individual mixograms of experimentally milled soft wheat flour samples from 11 states representing two crop years and a wide range in varietal quality and protein content (5.7 to 13.8%). The relation of linear measurements to area for the 344 samples is shown in Figure 1. Although the majority of points fall close to a regression line for all samples, there is a considerable number which deviate somewhat. It was found that the samples could be differentiated on the basis of mixing time and the ratio of the two linear measurements. Mixograms exhibiting mixing times of longer than one minute or ratios between height at point of minimum mobility and that at the 7-minute point of less than 1.68 were considered normal. This group included 306 samples.

Further study of the mixograms that were not classed as normal showed that they should be divided into two groups based on the ratio of the height at minimum mobility to that at the 7-minute point, namely those with ratios between 1.68 and 2.00 (34 samples) and those with ratios greater than 2.00 (4 samples).

The statistical data for all samples, as well as for each of the three groups, are as follows:

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Department of Agronomy, Ohio Agricultural Experiment Station.

[2] Junior Chemist, Division of Cereal Crops and Diseases at the Federal Soft Wheat Laboratory, Wooster, Ohio.

	Number	*Coefficient of correlation*	*Regression equation*
All samples	344	0.981	Y = 0.6132X + 5.462
Normal samples	306	.993	Y = 0.6345X + 3.555
Short mixing time samples			
Ratio 1.68–2.00	34	.992	Y = 0.5765X + 5.717
Ratio greater than 2.00	4	.995	Y = 0.5109X + 8.428

The differences between the correlation coefficients for all samples and those for each of the three groups have been tested and found to be very highly significant. Regression lines for the two special groups

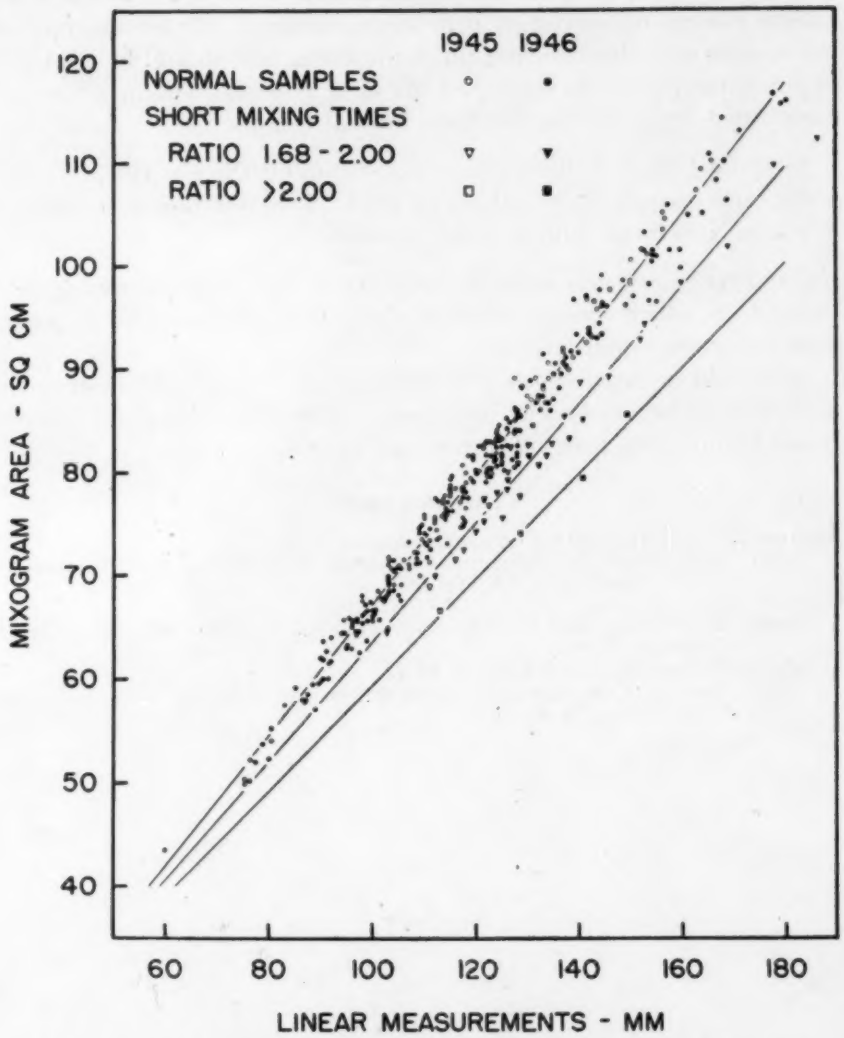

Fig. 1. Mixogram area and sum of linear measurement for 344 samples of soft winter wheat varieties grown at a number of locations and in two crop years.

represent two lower levels and have slopes which are slightly different from that of the normal samples.

The high correlation coefficient for all samples indicates that mixogram area can be predicted accurately from the two linear measurements. In addition, the improved correlation coefficients of the three separate groups compared to that for all samples indicate that by taking into consideration the length of the mixing time and measurement ratio in choosing the regression line, mixogram area can be predicted from the two linear measurements with additional accuracy.

The linear sum (L. S.) may be used as an index of soft wheat flour quality instead of converting it to mixogram area. In the instance of mixograms with short mixing times the linear sum should be adjusted to the same extent as suggested above for areas. This may be accomplished by correcting the linear sum as follows:

for ratio 1.68 − 2.00, corrected L. S. = $0.9086X + 0.3407$,
for ratio greater than 2.00, corrected L. S. = $0.8052X + 0.7680$,
where X = linear sum actually obtained.

If linear measurements are to be used extensively, tables showing corrected L. S. which correspond to various values of X may be prepared from the above equations.

It should be pointed out that, with the exception of Clarkan, relatively few commercial soft winter varieties consistently produce mixograms with mixing times of one minute or less.

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BOOK REVIEWS

Methods of Vitamin Assay. Prepared and edited by the Association of Vitamin Chemists, Inc. 189 pp. Interscience Publishers, Inc., New York, N. Y. 1947. Price $3.50.

The result of a cooperative effort of some 35 chemists, this volume elucidates the principles and practice of modern nonanimal vitamin assay methods in a thorough and intelligible manner. It is apparent that the majority of the authors have had detailed, firsthand experience with the procedures. Among the 12 methods for the determination of a total of five vitamins and one vitamin precursor will be found examples of virtually all of the basic techniques in current use. These are colorimetric (vitamin A, carotene, ascorbic acid), spectrophotometric (vitamin A), chromatographic (carotene), solvent partition (carotene), fluorometric (thiamine, riboflavin), fermentometric (thiamine), microbiological (riboflavin, niacin), and titrimetric (ascorbic acid).

Individual methods are presented in a detailed fashion not to be found either in the original publications in scientific periodicals, or in the *Official* methods of the U.S.P. and A.O.A.C. The basic principles and the rationale behind many of the precautions are provided and there is a liberal use of very valuable explanatory interpolations. The authors have stressed the applicability of the procedures to specific foods, feedstuffs, and pharmaceuticals. Where necessary, modifications of the extraction procedures, etc., are introduced. In this connection, it is hoped that the reviewer will be pardoned if he alludes to the obvious—technically, *Official* procedures are *official* only when applied to certain specified substances. Thus, the assay of the vitamin content of distillers' solubles, for example, by an Official method (U.S.P. or A.O.A.C.) is without any true official standing and, more important, is as likely as not to be in serious error. Therefore, although the procedures in *Methods of Vitamin Assay* are in no sense official, they probably deserve equal rating in many applications and may actually be superior in some instances.

Also emphasized are the experimental pitfalls which lie in wait for the uninitiated or unwary. It is worth mention that current vitamin assay procedures are not simple or easy, except by comparison with the older methods employing experimental animals.

The initial chapter deals with sampling for analysis. It is thoughtfully organized and, following a discussion of general principles, gives detailed consideration to (a) Meats and Other Animal Tissues, (b) Pharmaceuticals, (c) Cereals and Cereal Products and Mixed Feeds, (d) Fruits and Vegetables, and (e) Blood and Urine. The protection of material from the influence of light, heat, and moisture during and after sampling might have received more attention than it does. The preservation of samples for later recheck of the analysis might also be worthy of consideration.

The six succeeding chapters are devoted to the individual vitamins. A selected bibliography of methods for the vitamins not covered in the present volume constitutes the eighth chapter. The ninth and final chapter deals with the use of check samples in the control of vitamin methods and preparations that have been made by the Association of Vitamin Chemists for the distribution of such samples. This is a worth-while service and, if widely used, will improve the quality of both research and routine vitamin assays.

It is to be regretted that the book is relatively limited in scope, covering as it does only five vitamins, but perhaps, as the authors have indicated, this is largely due to the present state of the science. In any case, the present volume can hardly fail to be a very useful addition to the library of any laboratory presently engaged in vitamin assay, or contemplating the same.

In reading the various procedures, this reviewer was reminded of the practice so common during the years in which these methods were being developed. Many of us found that the surest and quickest way to pick up new methods was to visit the laboratory which had pioneered in the particular method and to look over the shoulder of a chemist as he actually made the determination. In a sense, the reader of *Methods of Vitamin Assay* is looking over the shoulder of 35 such chemists. This impression is based on the completeness of the presentation and the attention given to apparently small modifications which, in the words of the authors, are frequently so slight as to seem unworthy of publication, yet, when available, greatly increase the usefulness of the method. Another factor contributing to this impression is the mention, without

prejudice, of specific instruments and sources of reagents that have been found suitable.

The typography and binding of the book are excellent.

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Modern Cereal Chemistry. Fourth Edition, by D. W. Kent-Jones and A. J. Amos. 651 pp. The Northern Publishing Co., Ltd., Liverpool, England. 1947. Price $15.00.

Modern Cereal Chemistry has become of recognized value in the chemical literature; consequently, the revisions and additions which appear in the new fourth edition will be welcomed by cereal chemists generally. Dr. A. J. Amos, who contributed much to the third edition, appears as joint author of the present volume.

One of the principal reasons for the popularity of past editions of *Modern Cereal Chemistry* is that it has covered adequately the entire field of cereal chemistry. The new edition continues the policy of presenting the most complete coverage of cereal technology to be found in the English language. The book is particularly valuable because it brings together a combination of selected references to the cereal literature plus the stimulating discussions and evaluations by the authors of controversial topics. The work is much more than a compilation and review of the literature, although in this latter respect it is outstandingly complete. Literature references include articles published during 1946.

The new edition has larger pages than the former edition and space has been conserved by more concise arrangement of material. Thus, although the new book has fewer pages it contains two more chapters than the previous edition. Most of the chapter titles have been reworded slightly, but the subjects covered remain unchanged in general. It is particularly appropriate that one of the new chapters deals with vitamin assay. Barley is now treated in a separate section and a few pertinent facts on soybeans and potatoes are included in the chapter entitled, "Rye, Oats, Maize, Rice, Soya and Potatoes."

Chapter V, entitled "Some Physico-Chemical Aspects of Flour," has been expanded to include a discussion of oxidation-reduction potentials. The theory is presented as well as the significance of rH values in doughs. Likewise Chapter VI on flour strength brings together in a concise and clear manner the important concepts bearing on the subject. Also, most cereal chemists will likely be pleased by the authors' matter-of-fact discussion of wheat conditioning as presented in Chapter VII. The need for more exact information on wheat conditioning is made evident by the authors.

Cereal chemists on this side of the Atlantic will note with interest that time has brought no change of opinion from our British contemporaries regarding the usefulness of the standard baking test of the American Association of Cereal Chemists. This baking procedure has never been acceptable to European chemists, and for that matter it was probably overpopularized in North America. However, neither are the European baking methods entirely applicable elsewhere; consequently, some of the material in Chapter IX, "The Technique and the Chemistry of the Baking Process," will prove of only cursory interest in North America, but the authors have brought together interesting and stimulating material in this chapter.

As is true of earlier editions, there are chapters on the use of wheat and flour for special purposes and on the nutritive value of cereals. The facts contained in these chapters are readily available through other sources; consequently, their chief value in the book is that the material is at hand in a volume dealing with cereal chemistry. The same argument applies to Chapter XIV, "Cereal and Balanced Rations for Livestock." In contrast, the information presented on the microbiology of cereals is especially valuable because it brings to the attention of cereal chemists facts that are not readily available elsewhere.

The chapter on analytical methods will prove of interest because of the discussions that accompany the procedures and the incorporation of methods not used regularly in North America. Methods for vitamin assay of cereals have been placed in a separate chapter. This should be a convenience.

Modern Cereal Chemistry is a book that can be most highly recommended. The authors deserve the thanks of cereal chemists everywhere for preparing a new edition

when much of the work had to be done under the trying conditions that prevailed in England during the war and postwar periods.

However, one cannot but wish that circumstances had permitted the preparation of neater diagrams throughout the book. Also, it is very unfortunate that the material presented in Chapter II, "Principal Wheats of the World," was not brought up to date. Much of the data is no more recent than 1936. In the light of the production changes which the war years caused, there is little justification for devoting space to obsolete crop production data in a book on cereal chemistry.

The book has been carefully printed and the type is easy to read. The publishers have done only a fair job of binding.

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The Chemical Composition of Foods. By R. A. McCance and E. M. Widdowson. Second Edition, revised and enlarged. Chemical Publishing Co., Inc., Brooklyn, New York. 1947. Price $3.75.

This book contains a compilation of analyses of 609 different foods. The tables are given in two separate sections: in the first the composition of foods is presented in terms of grams or milligrams per 100 grams; in the second section in terms of grams or milligrams per ounce. Data are given for water, sugar, starch, total nitrogen, protein, available carbohydrate, calories, sodium, potassium, calcium, magnesium, iron, copper, phosphorus, sulfur, chloride, and acid-base balance. For fruits and vegetables data are also included for unavailable carbohydrate; for meat, poultry, game, and fish, values for "purine nitrogen" are listed. A separate table presents the phytic acid phosphorus content of about 60 foods. No data are given for the vitamin content of the foods listed. With few exceptions the analytical values reported apply to the edible portion of the cooked food; hence they can be applied directly to the calculation of dietaries. In case of cooked dishes containing several ingredients the recipes for these dishes are given.

A unique feature of these tables of food composition is the fact that they are not assembled from miscellaneous data in the literature; instead they are based on data accumulated in the authors' laboratory over a period of more than 20 years.

In the expression of analytical values and in calculation of calorific value of the foods, the conventions adopted are in some respects different from those commonly used in the American literature. Thus the factor used for the caloric value of carbohydrate is 3.75 because the analytical results are expressed in terms of glucose or of invert sugar; the caloric values for fat and protein are set at 9.3 and 4.1 respectively. The values for acid-base balance of foods are given in terms of $N/10$ acid or base, while in the American literature they are usually given in terms of $N/1$ acid or base.

A few random comparisons made between values given by McCance and Widdowson and those of a widely used American source show at times surprising discrepancies; thus the caloric value for butter given in the charts compiled by the H. J. Heinz Company, 12th Edition 1946, is 733 per 100 grams, while McCance and Widdowson give a figure of 793 Calories. Similarly the caloric value for doughnuts is given in these two sources as 425 and 355 respectively. These examples are cited merely to emphasize that the calculation of dietaries from analytical values in the literature is at best an approximation. For practical dietetics where analysis of the food consumed is usually impossible, such charts as those presented by McCance and Widdowson are of greatest value. *The Chemical Composition of Foods* should be particularly helpful to dieticians, physicians, and home economists.

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Fatty Acids—Their Chemistry and Physical Properties. By Klare S. Markley. x + 668 pp., 15 × 23 cm. Interscience Publishers, Inc., New York, N. Y. 1947. Price $10.00.

As one of a series of monographs on the chemistry and technology of the fats, oils, and related substances (Editorial Board consisting of A. E. Bailey, T. P. Hilditch,

H. E. Longenecker, and K. S. Markley), the purpose of this volume is to provide a comprehensive survey, in an organized and easily accessible form, of the present accumulation of facts and data pertaining to the chemical reactions and physical properties of the saturated, unsaturated, and substituted fatty acids. Particular emphasis is given those acids which naturally occur in the fats and other lipides.

Subject matter, pertinent data, and generally adequate discussions are organized into sections and chapters under headings that aid greatly in making the material readily accessible. An author index (15 pp.) and a subject index (20 pp.) serve to amplify this accessibility. There are 81 graphs and 163 tables of data together with nearly 1500 literature citations. The breadth of coverage is indicated in the following summary of the contents.

A short introductory chapter (11 pp.) on the history and nature of fats and waxes is followed by two chapters (63 pp.) on the classification, nomenclature, and isomerism of the fatty acids. The first major section, which follows, contains five chapters (165 pp.) in which the physical properties of the fatty acids are treated under the headings: crystal properties, spectral properties, thermal properties, solubility of fatty acids and solution properties, and properties of fatty acids in the liquid state. A second major section deals with the chemical reactions of the fatty acids and consists of 11 chapters (277 pp.). Included are extensive discussions on esterification and interesterification, pyrolysis, halogenation, hydrogenation and hydrogenolysis, oxidation and hydroxylation, autooxidation, and the nitrogen derivatives of aliphatic acids, together with short chapters on the salts of fatty acids, alkylation and alkoxylation, biological oxidation, and sulfur derivatives of the fatty acids. The next section of two chapters (43 pp.) is devoted to a fairly comprehensive discussion of the *in vitro* synthesis of the fatty acids and a consideration of the evidences for and against various theories concerned with the biosynthesis of fatty acids. The final section of two chapters (25 pp.) deals with analytical phases of fatty acid chemistry, namely, the separation of fatty acids and the identification of individual fatty acids.

The manner of organization, completeness of treatment, and coverage of subject matter are unique in this volume. It should find wide usefulness among chemists and technologists who are interested in the fatty acids, their products and by-products. Students will find it valuable as a source book of material fundamental to a study of lipide substances.

Few errors, either typographical or factual, were found.

D. R. Briggs
Division of Agricultural Biochemistry
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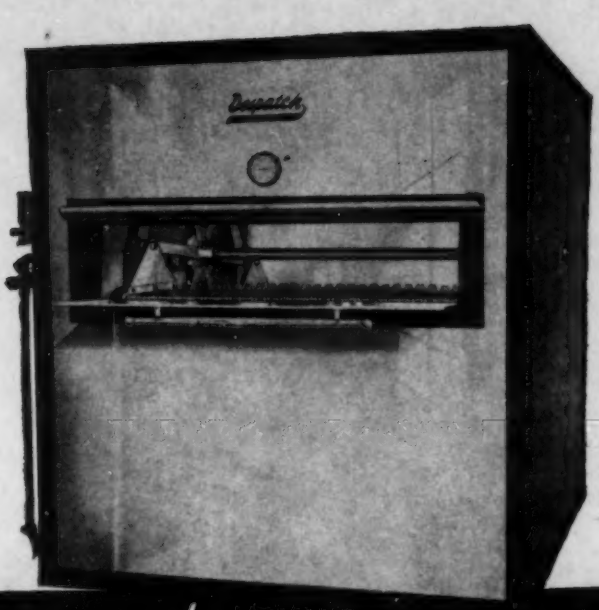

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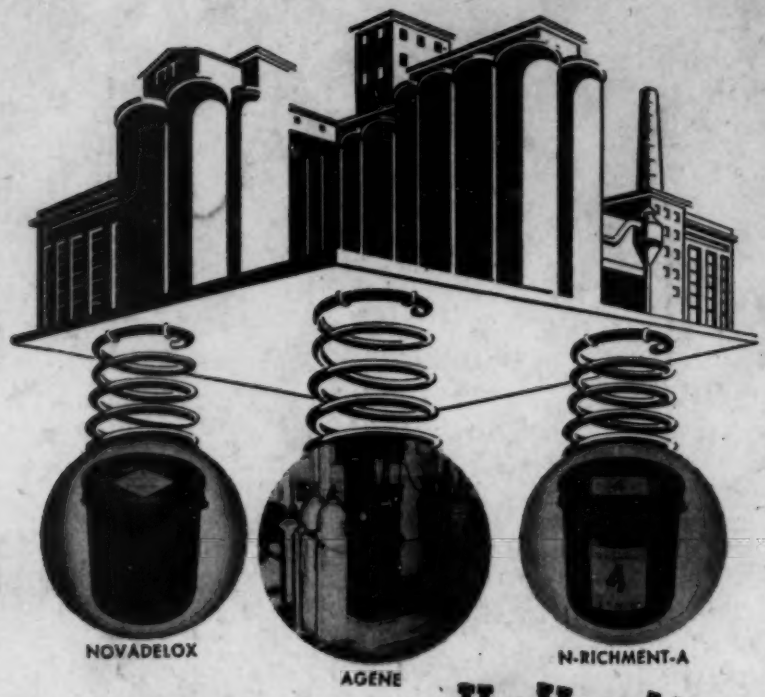

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